Science

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Recent Advances in Color Vision

H. Hartridge, F.R.S.

MANY THEORIES OF COLOR VISION have been suggested from time to time. Most of them have received little support, but two have been exceptions in this respect, namely, Young's three-color theory and Hering's four-color theory. The former is in apparent conformity with color mixture and with color deficiency data, while the latter is superficially in agreement with other important aspects of human color vision. The polychromatic theory to be dealt with in this address is the outcome of an attempt to combine together the good features of both of these older theories.

According to Young's theory human color vision is provided by a tricolor unit. On the other hand, according to Hering's theory it is provided by two units: a yellow-blue unit and a red-bluegreen-red unit. The polychromatic theory proposed by the author adopts the tricolor unit as its principal agent and the yellow-blue and red-bluegreen-red units as its subsidiary agents. Thus, human color vision is nearly trichromatic but not quite, because in addition to the tricolor unit there are also present the two additional units just mentioned.

Here, then, is an outline of the polychromatic theory.

Polychromatic theories have been advanced by Wundt, by Edridge-Green, and in a modified form by Shaxby. Granit's modulators also form, in some animals, what amount to a polychromatic series. The polychromatic theory advanced by the author differs from all these in important respects, and it is not proposed to deal further with them here, but instead to describe briefly the circumstances which lead to the development of this particular theory.

Until a few years ago experimental work appeared to support unequivocally the three-color theory of Thomas Young. When the author first observed the change of yellow to pale gray or white, and of blue to dark gray or black, which play so essential a part in the antichromatic responses, he endeavored, apparently with success, to account for them on this classic theory. But further facts then came to light incompatible with that theory. These facts were: (1) the change in the shape of the luminosity curve with reduction of light intensity, and the development of a notch (*26*); (2) the corresponding change in the shape of the luminosity curve with reduction of visual angle (*33*); (3) the finding by the author (*9*) of more fixation points in the fovea than three; (4) the finding by the author of more specific colors than three, as the result of applying microstimulation to the retina in the presence of suitable conditioning stimuli. These results, incompatible with the three-color theory, led to a re-examination of the whole subject of color perception.

The outcome of that survey convinced the author that, under average conditions of lighting, adaptation and field size, the three-color theory is a useful approximation so far as foveal vision is concerned. It is the truth but not the whole truth, because it is not in accord with a large mass of evidence, much of which has been obtained recently, partly by using improvements on older methods, such as the mixture of spectral colors by means of the Wright spectroscope, and partly by the development of entirely new methods—for example, the Stiles and Crawford (*30*) technique. But much older work was also found at variance with the three-color theory. When all this evidence has been taken into account, it would not be unfair to sum up by stating that there is hardly a single phase of human color vision which does not support a polychromatic theory more strongly than it does one based only on three colors.

I. The Polychromatic Theory

The polychromatic theory postulates the existence of two subsidiary visual units in addition to the tricolor unit of Young's theory. One of these, the Y-B unit, has receptors with response curves having crests in the yellow and in the blue parts of the spectrum. The other unit, the R-BG-R unit, also comprises two types of receptors, and these have response curves with crests in the red and in the blue-green part of the spectrum, but the former have, in addition, secondary curves in the extreme violet part of the spectrum. This fact is indicated by the duplication of R in the title of the unit.

When the fovea is being used in normal color vision, all three units are in action. When a more peripheral part of the retina is in use, the Y-B unit alone provides color vision. The fovea at very high light intensities also employs this unit alone, whereas at low intensities

This address by Prof. H. Hartridge, president of Section I (Physiology) of the British Association for the Advancement of Science, was delivered on September 13 during the Association's meeting at Brighton.

or small visual angles the R-BG-R unit functions alone. With white stimuli, all the constituent receptors of the functioning units receive adequate stimulation. When, on the other hand, the objects under examination are colored, the receptors which are stimulated are those which have response curves in the corresponding part or parts of the spectrum and which belong to functioning units. The following example may make this clearer: Suppose the ray incident on the retina to be red of 0.65 μ; then the stimulated receptors are the long wave-length receptor of the tricolor unit, the red receptor of the R-BG-R unit and the yellow receptor of the Y-B unit. If the ray which falls on the fovea is of medium light intensity, all three units will be potentially in action, and the sensation aroused by their combined actions will be a somewhat orange red.

At very high intensities, however, as stated above, the only active unit is the Y-B one, and in consequence the sensation aroused by the stimulus of wave-length 0.65 μ will be yellow. At low intensities the only active unit is the R-BG-R one, and the sensation aroused will consequently be a purplish crimson, since this is the hue produced by the activity of the red receptor of this unit which, as stated above, has response curves in both the red and the violet parts of the spectrum. These theoretical conclusions agree with experiment.

TABLE 1

Visual unit	Position of receptor in the spectrum	Probable wave length of crest of response curve*
Tricolor	Orange	0.610
"	Green	0.545
"	Indigo	0.450
Y-B	Yellow	0.575
"	Blue	0.473
R-BG-R	Red and violet	0.670 0.360
"	Blue-green	0.495

* These wave lengths are approximate only.

These three units together comprise 7 different types of receptor, and these have 8 response curves, the spectral positions of which are indicated in Table 1.

When white light falls on the retina, it stimulates all receptors of active units. Thus, at the fovea under medium light intensities all 7 receptors will be active, at high intensities the yellow and the blue receptors only, at low intensities the red and the blue-green receptors only. At the dichromatic zone of the periphery of the retina the yellow and the blue receptors are those which are stimulated, because the Y-B unit is the only one which is potentially active. This accounts for the large sizes of the yellow or blue fie in comparison with those of red and green. It clear, therefore, that according to the present po chromatic theory all 7 types of receptor are in only at medium light intensities at the fovea and parafovea. At other intensities and in the periphe of the retina a simpler receptor arrangement is in As the visual angle is reduced, first the Y-B unit b comes inactive, then the tricolor unit, finally leavi the R-BG-R unit alone in action. The author has pr duced evidence that these changes are not limited the foveal center but take place in all parts of t retina where all three units are normally in actio

II. Hue Discrimination

It will be remembered that there are two classes subjects who are able to pass successfully the ordina tests for color blindness—the normal trichromats a the anomalous trichromats. The latter are subdivid into two classes according as they require more red more green than the normal sighted, in order that red-green mixture shall match a given yellow. Tho who require more red and are therefore somewhat r blind are called protanomalous, since they appro mate the completely red blind or protanopic. Tho who require more green and are therefore somew green blind are called deuteranomalous, since th approximate the deuteranopic subject.

There are two varieties of protanomalous trichro —a primitive type and a more advanced type. T hue discrimination curve of the former consists two U-shaped parts having troughs at about 0.49 a 0.59 μ. To account for this curve three types of r ceptor are required, namely, in the long, medium, a short wave region of the spectrum. The hue discri nation curve of the better type of protanomalous su ject, such as Wright's observer A, shows improvem in the green part of the spectrum. To account f this improvement, an additional receptor is requir probably in the blue-green part of the spectrum. I vestigation shows that an alteration of position, o change of width, of the response curve of one of t three receptors mentioned above does not bring ab the improvement shown on comparing the curves.

The color vision of the normal subject as found Pitt and Wright (1934) shows improvement at ab 0.44 μ over that possessed by observer A, and there a second region of improvement near the orange p of the spectrum. To account for these improvem two additional types of receptors are required, in t blue and the yellow. Finally, some normal-sight observers, such as Steindler (*27*), Jones (*16*), a Laurens and Hamilton (*20*), appear to have bet hue discrimination still, particularly in the red p

of the spectrum. This calls for an additional type of receptor.

Calculations of the hue discrimination curve of normal-sighted subjects have been made by Helmholtz (*11*), Judd (*17*), Stiles (*29*), and others, on the basis of the three-color theory. These calculated curves are far from being identical in shape with the experimental curves obtained with normal-sighted, protanomalous (including Wright's observer A), and deuteranomalous observers, as should be the case because, according to the three-color theory, all these three classes are 'trichromats.' Thus, the three-color theory fails to give a satisfactory account of the hue discrimination of these subjects. The polychromatic theory, on the other hand, postulates 3 types of receptor for the more primitive protanomalous subject, 4 types for the more advanced type, probably 5 types for the deuteranomalous, and 7 types for subjects with normal color vision.

III. Tristimulus Spectral Mixture

It is well-known that when the different parts of the spectrum are matched using as stimuli a red of 0.65 μ, a green of 0.53 μ, and a blue of 0.46 μ, trichromats usually find that most of the spectral colors are more saturated than the mixtures. These differences of saturation are noticeable in four main spectral regions: in the red near the long wave end of the spectrum, in the yellow, in the blue-green, and in the violet near the short wave end of the spectrum.

Trichromats differ markedly from one another in their ability to match the rays corresponding to these spectral regions with the three matching stimuli. These differences, so far as the yellow and blue-green are concerned, are summarized in Table 2.

In Table 2 the various classes of trichromat are arranged in order, the more primitive protanomalous subject first, because the differences in saturation of the monochromatic spectral colors and the mixed colors are very small, or absent. Then comes the improved kind of protanomalous subject such as Wright's observer A; then the fovea of the normal-sighted subject; then the parafovea of the normal-sighted subject; and lastly, the deuteranomalous subject to whom the blue-green spectral colors are far more saturated than the mixture of the green 0.53 μ and the blue 0.46 μ.

The differences in saturation between the monochromatic and the mixed colors are accounted for on the three-color theory by supposing that the three-receptor processes have poor spectral selectivity—that is, that their response curves spread over much of the spectrum. Thus, the blue response mechanism is sensitive to almost all greenish rays as well as to blue ones; the green response mechanism is sensitive to almost all rays; and the red response mechanism is sensitive to almost all green rays, as well as to yellow, orange, and red ones. In consequence, monochromatic yellow light, which acts as a stimulus to both the red and the green response mechanisms, but not to the blue ones, appears, according to this hypothesis, to be more saturated than a red and green mixture which stimulates not only the red and the green mechanisms but the blue ones as well.

In support of this hypothesis MacAdam (*22*) has pointed out that, when reproduced by a three-color photographic process, spectral yellow is more saturated than a red-green mixture. In this case there can be no doubt that the wide overlap of the response curves of the three color-selecting filters is responsible for the observed differences of saturation.

Suppose that this argument be accepted in the case of human color vision. It is then found to lead to the conclusion that subjects who do not find differences of saturation between monochromatic and mixed colors—

TABLE 2

Type of subject	Negative coefficients	
	Red stimulus in blue-green part of spectrum	Blue stimulus in yellow part of spectrum
Normal protanomalous	Nil	Nil
Improved protanomalous	2–3%	Nil
Normal fovea	10–12%	3–5%
" parafovea	10–12%	10–12%
Deuteranomalous	Over 12%	Under 3%

that is, subjects who are protanomalous trichromats—either have receptors of narrower response (*i.e.* of greater selectivity) than other trichromats, or that their saturation discrimination is poor, so that they make matches which would be unacceptable to normal subjects who are better in this respect. Now Chapanis (*4*) has shown that the latter is not true, because, so far as the yellow region of the spectrum is concerned, the protanomalous possess better saturation discrimination than the normal-sighted do. It is therefore concluded that their receptors have better selectivity because the response curves of these receptors do not overlap as much as they do in the normal sighted. On comparing similarly protanomalous with deuteranomalous trichromats it is concluded that the former have receptors with much greater selectivity than those of the latter. Now receptor selectivity plays a fundamental part in color vision because poor selectivity must cause all colors to look much alike, so that no color approaches in saturation that which would be produced by the stimulation of a single type

of receptor only. Vice versa, high receptor selectivity must cause colors to appear to possess a high degree of saturation. On these grounds, of all the types of subject with trichromatic vision mentioned in Table 2, the protanomalous must have the best color vision and the deuteranomalous the worst. The normal-sighted come in between, with the fovea superior to the parafovea. Now these conclusions are completely at variance with the facts, for the protanomalous are little better or worse than the deuteranomalous, and both are greatly inferior to the normal-sighted, in whom the parafovea is superior to the fovea.

According to the polychromatic theory the monochromatic colors have superior saturation to the mixed colors because the former stimulate an additional receptor more strongly than do the latter. Thus, to the normal-sighted subject spectral yellow is more saturated than a red-green mixture, because the former stimulates yellow receptors strongly and red ones and green ones feebly. On the contrary, the red-green mixture stimulates the red and green receptors strongly but the yellow ones feebly. In a similar manner enhanced saturation of the monochromatic spectral colors in the extreme red is caused by the additional type of receptor which operates in this part of the spectrum. Additional blue-green receptors improve saturation in the blue-green region, and additional violet receptors in like manner improve short-wave saturation. Since, to the primitive type of protanomalous subject, the single spectral colors and the spectral mixtures which match them are alike in saturation, no additional receptors are present, and their color vision is mediated by the fundamental tricolor unit alone. Thus, of all so-called trichromats, they should have the most inferior color perception. The improved type of protanomalous subject, to whom the blue-green rays are more saturated than are blue and green mixtures, has the additional blue-green type of receptor according to the polychromatic theory, and therefore is superior to the more primitive protanomalous subject in this respect. The position of the deuteranomalous subject is not entirely clear, but according to present evidence he possesses both the tricolor unit and the blue-green and possibly also the red receptors of the R-BG-R unit. But the former is outweighed by the latter so that he approximates a dichromat, and this accounts for his inferior color vision. For normal foveal vision 7 types of receptor are required, the three-color unit and the four additional receptors of the Y-B and the R-BG-R unit, the former strongly and the two latter, particularly the Y-B unit, feebly. At the parafovea, so far as present evidence goes, the same 7 types of receptor are present, but the additional ones hold a more prominent position, so that they play more nearly the same part as that played by the tricolor unit.

According to the polychromatic theory, therefore, both the protanomalous and the deuteranomalous have inferior color vision to the normal-sighted subject; in the latter, the parafovea has superior color vision to the fovea, and these deductions are found to be in harmony with the results of experiment.

IV. The Retinal Direction Effect

When a narrow pencil of monochromatic light traversed the pupil horizontally, Stiles (*28*) found changes in brightness, hue, and saturation. The author (*9*) has pointed out that the magnitude of the brightness effect for yellow rays is substantially less than it is for either red or green rays. This makes it unlikely that yellow rays stimulate red and green receptors alone, as is postulated by the three-color theory. It is more probable that there are specific yellow receptors having a different intensity direction effect from that of the red or green receptors. The other aspects of the retinal effects on intensity can be accounted for on the three-color theory.

Stiles (*28*) found that the variations of hue with wave length are more complex than would be expected on the three-color theory. On this theory there are only 8 possible combinations, of which the two exhibiting the greatest variation with wave length are red +, green −, blue +, or vice versa: red −, green +, and blue − (that a single crest in the green with lower values in the red and in the blue, or a single trough in the green with higher values in the red and the blue). Now Stiles obtained on the temporal side of the pupil a graph exhibiting two troughs and one crest, whereas on the nasal side the graph showed more detailed changes still, namely, two troughs and two crests. In order to account for the former a minimum of four types of receptor are required; in order to account for the latter a minimum of five types. Unfortunately, Stiles has not investigated the phenomenon with sufficient detail for a more precise estimate to be made of the number of types of receptor required, but there would seem to be no difficulty in doing this.

With regard to the third retinal direction effect, namely, on saturation, Stiles has not given any details of this beyond mentioning the fact that such variations exist. No statement is possible at the moment, therefore, as to its value in the elucidation of the problems of color vision. The effects on brightness and hue may be summarized as follows: the brightness effect requires at least four receptors, of which yellow must be one; the hue effect requires at least five.

urther study of the effects may show the necessity r more.

V. Peripheral Vision and Reduced Foveal Vision

The term 'reduced foveal vision' has been used by e author (*9*) to denote the type of hue perception ound at the fovea at low light intensities and at mall visual angles. Reduction of intensity or visual ngles results in the replacement of full color vision, rst by a form of dichromatism and then by colorless oveal vision. In the dichromatic stage, colors are en in terms of two primaries only, red and blue-reen, and mixtures of these with white, gray, or lack. The author attempted to account for this type f vision on Young's theory by supposing a type of nkage to occur between the green and the blue re-eptor pathways, but this supposition had to be bandoned when the changes in the shapes of the uminosity curves found by Sloan (*26*) and Willmer nd Wright (*33*) were taken into account. During e changes just referred to, many colors, both spec-al and mixed, undergo alterations in hue, but there re four invariable colors: blue, blue-green, yellow, nd red. The blue and the yellow are replaced by olorless shades of gray at an early stage of the hange; the blue-green and red are similarly replaced y grays, but at one of the last stages.

When foveal vision is gradually replaced by peri-heral vision, color perception becomes reduced until, s in the case of foveal vision, a colorless state is btained. At an intermediate stage in this change ichromatic vision occurs in which colors are seen in erms of yellow and blue and mixtures of these with hite, gray, or black. In this case, also, four in-ariable colors are found (blue, blue-green, yellow, nd red), but whereas with reduced foveal vision the ellow and the blue suffer early replacement and the lue-green and the red suffer later replacement by rays, in this case of peripheral vision the situation is eversed. Attempts have been made to account for hese changes in color vision on Young's theory, by upposing that linking occurs between the red and he green receptor pathways. Thus, the vision of the lue-yellow zone of the periphery is identified with he dichromatic vision of the deuteranopic subject in hom permanent linkage of these pathways is sup-osed to be present. But this supposition also is endered untenable by an examination of the liminos-ty curves, because that of the dichromatic zone of he retinal periphery was discovered to have a crest t 0.59–0.60 μ (*19*). When these values were corrected or an equal energy spectrum by the author, the crest f the luminosity curve shifted to a wave length of bout 0.58–0.59 μ. Now the crest of the luminosity curve of the deuteranope, as found by Pitt (*23*), occurs at 0.56 μ. Thus, the suggestion of linking between certain receptor pathways has to be abandoned. These difficulties introduced by the liminosity curves are not the only ones making the position of the three-color theory untenable.

According to the polychromatic theory, with reduction of visual angle or lowering of light intensity, or both, the Y-B-unit is the first to become inactive, and this is followed by the tricolor unit. In consequence, the R-BG-R unit is the only one in a state of activity. The notches in the luminosity curve observed by Sloan (*26*) and by Willmer and Wright (*33*) are due to the inactivity of the Y-B unit in the blue and in the yellow parts of the spectrum. On the other hand, when a more peripheral part of the retina is employed, the first unit to become inactive is the R-BG-R, and this is followed by the tricolor unit. At this stage the only unit in action is the Y-B one, and this has the luminosity curve found by von Kries.

So far no mention has been made of the effects of increasing light intensity. The color changes as found by Purdy (*24*) strongly resemble those produced by using a peripheral part of the retina. If the illumination be sufficiently bright, there is colorless foveal vision; at a less intense illumination there is yellow-blue vision, which, according to the polychromatic theory, is due to the activity of the Y-B unit. Thus, the polychromatic theory accounts for these phases of color vision by postulating activity of certain visual units and the inactivity of others, and in this way accounts for their properties in a satisfactory manner.

VI. The Constancy of White

When identical pairs of colored test objects are examined, one by the fovea and the other by the periphery of the same eye, most of them are found to differ; yet when white test objects are similarly examined, no differences can be detected. When similar experiments are performed to test the effects of light intensity or of size of visual angle, colored test objects are found to undergo marked differences, but white test objects similarly tested are found to undergo no observable change. This constancy of white is the more remarkable because deviations from white are so readily noticed.

Now according to the three-color theory, the perception of white is due to the stimulation in a certain fixed ratio of all three receptor processes, so that the constancy of white proves that this ratio is the same for the periphery of the retina as it is for the fovea and is the same at the fovea for high light intensities as for low ones and for large visual angles as for small ones. The important color changes which

are found to occur under these different conditions make it imperative that there should be alterations in color perception, and yet the constancy of white shows that they cannot occur so far as the three-color theory is concerned.

While great difficulty is experienced on the three-color theory, no such difficulty faces the polychromatic theory, because it is the property of the three units, either separately or in combination, to respond to a white stimulus by means of a white sensation. Suppose, for sake of argument, that orange, green, indigo, and purple be examined (a) by the dichromatic zone of the retinal periphery where the Y-B unit is found in a pure state and (b) by the dichromatic vision of the fovea at a small visual angle which is mediated by the R-BG-R unit; then these colors will all be found to exhibit marked differences when examined first by the one type of vision and then by the other. But white similarly tested will be found to undergo no change, because, as stated above, both the Y-B and the R-BG-R units respond to a white light stimulus with a white sensation.

VII. The Effects of Light Intensity, Adaptation, and Visual Angle on Foveal Spectral Mixture

Various factors have been found to affect the matches between monochromatic spectral colors and mixtures of spectral colors. Thus, von Kries (*18*) investigated the effects of light intensity; Wright, the effects of adaptation (*34*); and Hering (*12*), Horner and Purslow (*14*), and Shaxby (*25*), the effects of visual angle on the match between yellow and a red-green mixture. These effects, recently confirmed by the author, are summarized in Table 3.

TABLE 3

	Red-green mixture compared to pure yellow
High intensity	Greener
Low "	Redder
Light adaptation	Greener
Dark "	Redder
Large visual angle	Greener
Small " "	Redder

The three-color theory, which postulates that yellow and a red-green mixture resemble one another in stimulating the red and the green receptors, finds it difficult, if not impossible, to account for these disturbances of color matches. Attempts to account for them on the grounds of the selective absorption of the yellow pigment of the fovea or of the greater "rod value" of green are both found to fail. According to the polychromatic theory, on the other hand, there are independent receptors for red as well as for orange, for yellow, and for green. The red ones, since they belong to the R-BG-R unit, play a more important part in foveal vision at low light intensities, at small visual angles, and when adapted to low light intensities. Thus, under these conditions the red component of the red-green mixture is accentuated, and this disturbs the match with yellow.

VIII. Selective Adaptation

Burch (*2, 3*) concluded from fatigue experiments that the human retina contains four types of receptor: red, green, blue, and violet. He was unable to obtain evidence for a fifth type of receptor, namely for yellow rays.

Allen (*1*), after fatiguing the eye to rays of 0.589 μ or of 0.577 μ, found that the rate of flicker was noticeably reduced both in the red and in the green but hardly at all between 0.64 μ and 0.58 μ. This was held to be evidence that yellow rays are perceived owing to the stimulation of red and green receptors but not of specific yellow receptors.

Edridge-Green and Marshall (*5*), after exposing the eye for a short time to a sodium lamp, claimed that they found unmistakable evidence for separate yellow receptors. The author (*10*), using yellow color filters, both monochromatic and dichromatic (red and green), of sharp spectral cut-off and equal visual appearance, found on fatiguing the eye that different results were obtained. After fatigue with the monochromatic yellow light the spectrum appeared to be greatly modified in appearance: yellow appeared neutral gray, red and orange appeared crimson, yellow-green and green appeared blue-green, and blue and violet were much darkened. On the other hand, exposure of the eye to the red and green mixture darkened the entire spectrum and produced slight selective effects on the colors of its different parts, but not of the same degree as those produced by the monochromatic yellow light. On the trichromatic theory it is difficult to explain why these two lights which resemble one another so closely should produce different fatigue effects. According to the polychromatic theory, on the contrary, the monochromatic yellow light stimulates the yellow receptors strongly and the orange, red, green, and blue-green receptors feebly. The dichromatic yellow light stimulates the red, orange, green, and blue-green receptors strongly and the yellow receptors feebly; hence, different results are achieved on subsequently examining a spectrum.

It remains briefly to refer to the curious inhibiting effect which exposure of the eye to monochromatic yellow light has on the subsequent perception of blue and violet, and that which exposure of the eye to blue

violet has on the subsequent perception of yellow. is is paralleled by similar effects when both are ated side by side in the same visual field, attention which has been directed previously by the author . It would appear possible that both simultaneous subsequent presentation operate in a similar manjust as simultaneous and successive contrast apr to do. It is hoped to deal elsewhere with this ject in greater detail.

IX. Saturation Discrimination

The apparent saturation of the spectral colors is turbed by actual or potential neutral points caused the response curves of the receptors occupying mplementary or nearly complementary positions in spectrum. Thus, for the protanope who possesses types of receptor only, which are complementary one another, there is an actual neutral point at out 0.49 μ.

Let us take a second example. To the fovea of the rmal subject when observing test objects at small ual angles, there is dichromatic vision probably due the activity of the R-BG-R unit in a pure state. ow the red and blue-green receptors are complemenry to one another, and in consequence there are two ual neutral points, at 0.578 μ and 0.41 μ (*33*).

Let us now take a third example. It was found Chapanis (*4*) that to the protanomalous subject spectrum at 0.49 μ is nearly neutral and there is second, not well-marked, potential neutral point at 56 μ. In these subjects, who have only three types receptor in a functional state, no one pair is complementary to any other pair, but the red and green ceptors produce the potential neutral point at 0.56 μ nd the two acting together produce with the blue ceptors the nearly neutral point at 0.49 μ. Now the icolor unit of the normal-sighted subject closely reembles, if it is not identical with, the visual unit of e protanomalous subject. If this be correct, the icolor unit is responsible for one nearly neutral int at 0.49 μ and for one point of poor saturation t 0.56 μ. Similarly, the Y-B units of the normalghted is found to have an actual neutral point at 526 μ, and, as we have seen, the R-BG-R unit posesses two actual neutral points at 0.578 μ and at 41 μ.

Taking these neutral points in turn, there are two the yellow, one caused by the R-BG-R unit and one by the tricolor unit. These correspond in wave-length the crests of the yellow receptors of the Y-B unit. ow this is only a subsidiary unit, so that the yellow ceptors modify but slightly the potential neutral oint produced by the other two units. The conequence is that the yellow region of the spectrum is a point of low saturation. The next neutral point in the spectrum is at 0.526 μ, due to the Y-B unit, but this is close to the crest of the important green receptors of the tricolor unit, the consequence being that this potential neutral point does not disclose its presence in normal color vision. In a similar manner the neutral point at 0.49 μ due to the tricolor unit coincides almost exactly with the crests of the blue-green receptors of the R-BG-R unit and is thus covered up. Lastly, the neutral point at 0.41 μ belonging to the R-BG-R unit is not far removed from the crests of the indigo receptors of the tricolor unit and is thus hidden by them. The conclusion is that of all these potential neutral points, the only one of consequence in normal vision is the one which occurs in the yellow part of the spectrum. This conclusion, which has been arrived at on the basis of the polychromatic theory, is in good agreement with the experimental data, because, as is well known, the point of lowest saturation in the spectrum is in the yellow. The three-color theory can offer no explanation of the variation of saturation with wave length exhibited by the protanomalous and the normal-sighted.

X. The Antichromatic Responses, the Fixation Points, and the Subjective Colors

Having reviewed in the previous sections some of aspects of human color vision for which the three-color theory is unable to provide a satisfactory account, but which can be explained on the polychromatic theory, it is now necessary to re-examine the experiments referred to in the introduction, which, being of a subjective nature, might not alone be considered to carry much weight, but materially add to the evidence given in the foregoing sections. The experiments referred to are those on the antichromatic responses, the separate fixation points of the different colors, and lastly, the subjective estimates of hue when, by means of a microstimulator and in the presence of conditioning stimuli, a tenuous beam of white light is caused to explore the retina.

Ever since the days of Thomas Young it has been agreed that the lens system of the eye suffers from chromatic aberration. The most recent measurements of the aberration at different wave lengths were made by Ivanoff (*15*) and by the author (*9*). Agreement between them is extremely good—so good, in fact, that further estimates on healthy emmotropic or nearly emmotropic subjects would appear to be unnecessary.

Now an examination by means of a microscope or high-power eyepiece of the images produced by uncolor-corrected lenses shows that color fringes are present at the borders separating the light and dark

parts of the pattern present in the visual field. These fringes at the optimum focusing point are yellow and blue. But it is a matter of everyday experience that their presence is seldom observed in normal vision. Experiments of a simple and reliable nature using blue and yellow test objects, those produced both by the use of dyes and color filters and by means of a suitable spectrometer, show that there is present in connection with vision a mechanism of some kind which can eliminate not only the colored fringes produced by the chromatic aberration of the eye but the colors of yellow and blue test objects as well. Experiment shows that many factors are concerned with this elimination—the intensity of illumination, the relative intensities of the spectral components present in the fringes, etc. Of special importance is the proximity of yellow and blue, because this is found to facilitate greatly the loss of hue by both colors. A number of different hypotheses have been advanced by the author to account for these facts, but one alone stands up to detailed examination, one, moreover, which is based on the polychromatic theory, namely, that the proximity of the blue and the yellow fringes, which is always found to occur when they are produced by chromatic aberration, aids the inactivity in the first place of the Y-B unit and in the second place of the tricolor unit, so that the only visual unit left in action is the R-BG-R unit. There is evidence supporting the view that these changes are brought about by a nervous mechanism which has been called by the author the antichromatic responses.

Some theories require, in a manner at present unknown, all color receptors to respond efficiently to all parts of the spectrum. As an example of these the theory advanced by Wright (*35*) may be quoted. He writes: "The triple sub-division may very possibly occur within the cones on a submicroscopic scale which cannot be revealed by our present techniques." Apart from these theories which postulate that all cones respond to all colors, the remaining theories suppose the receptors to be divided into a certain number of different types. In the case of the three-color theory there are obviously three types; in the case of the polychromatic theory, several types; and so on. Now these theories automatically require that there be different fixation points for different colors, since it is obviously impossible to suppose that a given receptor fixes all colors, both those which come within the range of its response curve and those which do not. Thus, in the case of the three-color theory there must be three different fixation points, one for red rays, one for green rays, and one for blue rays. A four-color theory will correspondingly require four fixation points and so on. Whatever the number may be, several different fixation points would be expected. Such an expectation is derived from first principles and is not directly dependent on the cluster hypothesis with which it is in complete accord, since the most likely situation for a fixation point, for a particular color, is near the center of a cluster of receptors which respond to that color.

It will be recollected that two different methods were employed by the author (*9*) when determining the positions of the fixation points in the foveal area: (a) the kinesthetic method, which depended on the subjective appreciation of eye movements, and (b) the local scotoma or blind-spot method. These methods gave confirmatory results. That local scotomata are present near the foveal center was first discovered by Franklin (*7*) and was supported by Wilbrand (*32*). That the position of the fixation point is a function of the wave length has been suggested by some experiments performed by Thomson (*31*). This conclusion is substantiated by observations made by the author. It is reasonable, therefore, to accept the hypothesis (a) that there are different fixation points for the different colors, (b) that their positions can be determined by suitable methods, (c) that their positions are a function of the wave length. The author decided that there were 7 different fixation points at a time when he was unaware of the large mass of evidence in favor of the polychromatic theory. These pieces of evidence therefore provide strong support for one another.

Probably the first experimenter to apply the principle of microstimulation to the human retina was Holmgren (*13*), who caused the image of a very small point of light to pass slowly over the surface of the fovea. When the source of light was yellow Holmgren found that it was seen as red in some positions and as green in others. It was thought at the time that this gave strong support to the three-color theory, but we now know, in view of the antichromatic responses, that this is not the case. In the same year Fick (*6*) found that if a source of colored light be sufficiently reduced in visual angle it becomes colorless. He also found that if two or more such sources of the same color be reduced in visual angle until, seen separately, they appear colorless, when seen together their colors are restored. These observations of Holmgren and Fick have been confirmed and extended by the author using the microstimulator in combination with a spectrometer for obtaining monochromatic light. On the basis of the three-color theory it would be expected that, when the image of a small source of white light is caused to move slowly over the surface of the fovea, it would

be seen as red in some positions, as green in other positions, and as blue in yet other positions. But in addition to these colors the author has also observed orange and blue-green. When suitable conditioning stimuli are present at the same time, as used by Fick, yellow and violet are also to be seen, making 7 colors in all. This is one more piece of evidence which supports the polychromatic theory.

XI. Some Other Receptor Arrangements

When alternative hypotheses to the polychromatic theory were examined by the author, particular attention was paid (a) to modifications of the three-color theory and (b) to three-channel hypotheses.

One modification of the three-color theory has already been referred to in this address namely, linking between the red and the green receptors at the fovea at high light intensities and at the periphery of the retina, and linking between the green and the blue receptors at the fovea at low light intensities and at small visual angles. Of all the modifications of the classical three-color theory this seemed to the author to be the most promising. It fails, however, mainly for three reasons: (a) it gives no explanation of the changes in shape of the luminosity curve; (b) it gives no adequate account of the differences of color vision between the true trichomats—that is, of subjects with normal sight on the one hand and between the protanomalous and the deuteranomalous on the other; (c) it does not comprise the necessary number of different types of receptor to comply with the requirements of the phases of vision referred to in sections II, III, IV, VII, VIII, and X. In consequence there is no alternative but to abandon this attractive hypothesis.

Of the three-channel hypothesis, one of the best known is that proposed by Granit himself (*8*) soon after his discovery in the mammalian retina of modulator response curves. His suggestion was that the nerve pathways from the orange and the yellow modulators might converge onto a single channel, that the three green modulators might do the same, and similarly for the two blue ones. Thus, there would be polychromatism in the retina but trichromatism in some part or parts of the nervous channels between the receptors and the brain, and possibly in the brain itself. Le Gros Clark (*21*), as the result of a histological survey of the external geniculate body of the monkey, has proposed that this structure forms a part of this triple channel. Now there can be little doubt that this hypothesis is in certain respects an improvement on the preceding one; thus, the polychromatism of the retina offers an explanation of the retinal direction effects which depend on local conditions. It also accounts for the many different fixation points. Important facts which it cannot deal with are spectral mixture, hue discrimination and the essential differences between the normal-sighted subject and the anomalous trichromat, all of whom must use the same three channels if this hypothesis is correct. Apart from these general criticisms there are some special ones, because Le Gros Clark has claimed that the periphery of the retina is dichromatic, with yellow and blue as primary colors. But this is only true at moderate intensities of illumination and ceases to be so if the light intensity is increased. He has also claimed that the fovea is dichromatic, with red and blue-green as primary colors. But in this case also the dichromasy is conditioned by light intensity and disappears, if this be increased. Lastly, the external geniculate body, if Le Gros Clark's description is correct, contains no provision for a channel connecting the rod receptors with the brain. If this does not pass through the external geniculate body, what course does it pursue? These are but a few of the difficulties which beset this three-channel theory.

Of four-color theories, the only one which need claim attention here is that originally proposed by Hering (*12*), because it is representative of the others. The four most likely receptor types have response curves with crests in the red, the yellow, the blue-green, and the blue, but the red receptor also has a subsidiary response in the violet part of the spectrum; in consequence the four types of receptors comprise five response curves. Since the red and the blue-green receptors are complementary, and since the former also acts in the violet, they form a unit which may be identified as the R-BG-R unit of the polychromatic theory. In the same way the yellow and blue receptors of Hering's theory, since they are complementary, may be identified as the Y-B unit of the polychromatic theory. Thus, Hering's theory in a modern form may be regarded as similar to the polychromatic theory, from which the tricolor unit has been omitted. What, then, would be the disadvantages of doing this? For normal foveal vision both units would be in operation. On reducing light intensity or visual angle the Y-B unit would drop out, leaving the R-BG-R unit alone in action. At high light intensities the Y-B unit would be the only effective one. The same unit would operate when there is dichromasy of the retinal periphery. Since these units comprise complementary pairs of receptors, the constancy of white under all these conditions is satisfactorily accounted for. The varieties of color blindness are not so readily explained. Protanopia might be due to the Y-B unit, and deuteranopia to linkage of the yellow and the red receptors to form a single receptor mechanism together with the

corresponding linkage of the blue-green and the blue receptors to form the second receptor mechanism. Protanomaly might be caused by linkage of the red and the yellow receptors, the green ones and the blue ones acting separately. Deuteranomaly might be caused by linkage of the green and the blue, the red and the yellow receptors acting separately, and so on. Where the four-color theory fails most obviously is when it attempts to fit in with hue discrimination data.

An examination of other theories leads to the final conclusion that only the polychromatic theory is in agreement with all the known facts, and that this not only must extend along the nervous channels which connect the receptors to the brain but must also apply to the brain itself.

Summary

Until a few years ago it was possible to account for nearly all the aspects of human color perception on the basis of the three-color theory, but such is no longer the case. This is largely due to improvements in the older methods of investigation and to the invention of new ones. Among the latter may be mentioned the microelectrode technique of Granit and the retinal direction effect of Stiles and Crawford. Modern requirements are met by a polychromatic theory, comprising 7 types of receptor, but there is no necessity for these to have such narrow spectral response curves as those exhibited by Granit's modulators. Modifications of the three- and four-color theories have been examined to see to what extent they can be made to fit in with experimental results. Particular notice has been taken of the possibility that there is polychromatism of the retinal receptors, but trichromatism of the nerve paths which connect these to the brain or even of the brain itself.

The conclusion arrived at is that there must be polychromatism throughout the entire visual mechanism for color perception if a complete account is to be given of all the known facts.

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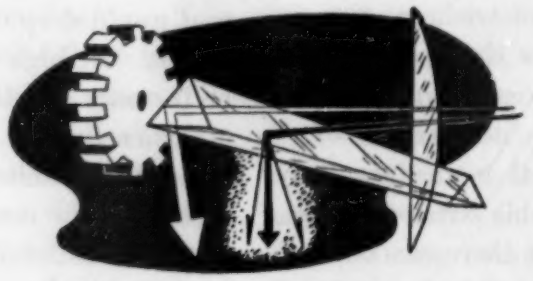

NEWS and Notes

AAAS Publications

The AAAS Executive Committee has given serious and extended consideration to filling the editorial positions left vacant on *Science*, following the death of Dr. W. L. Valentine in April 1947, and on *The Scientific Monthly*, following the resignation of Dr. F. L. Campbell this past spring. It has been a difficult problem to make suitable appointments for these important positions under present conditions of manpower shortage and the prevailing high salaries.

At the July meeting of the Executive Committee, it was unanimously voted to appoint an Editorial Board of 5 members for the AAAS publications to serve for one year, together with a Chairman who would act also as Editor-in-Chief. The Editorial Board as now organized to cover the various scientific fields consists of Arthur C. Bevan, of the Illinois State Geological Survey Division, Edward U. Condon, of the National Bureau of Standards, H. Bentley Glass, of Johns Hopkins University, Malcolm H. Soule, of the University of Michigan, and Everett S. Wallis, of Princeton University, with George A. Baitsell, of Yale University, as Chairman. Yale University has granted temporary half-time leave of absence to Dr. Baitsell to permit him to render this service to the Association.—EDMUND W. SINNOTT, *President, AAAS.*

About People

William L. **Everitt**, head of the Department of Electrical Engineering, University of Illinois, since 1944, will succeed Melvin L. **Enger** as dean of the College of Engineering and director of the Engineering Experiment Station upon the latter's retirement on September 1, 1949.

Rev. Eugene A. Gisel is the new head of the Department of Chemistry at Fordham University. During the recent war Father Gisel, who had been teaching chemistry at the Jesuit College in Manila, was taken prisoner by the Japanese. Upon his return to this country he became head of the Chemistry Department at St. Peter's College, Jersey City.

Anton J. Carlson, professor emeritus of physiology, University of Chicago, will deliver the second annual Margaret Barclay Wilson Memorial Lecture in the North Lounge of Hunter College, 695 Park Avenue, New York City, at 4:15 P.M. on October 19. Dr. Carlson will speak on "Diet and the Span of Life."

Joseph P. Maxfield, after a 31-year association with the Bell Telephone Laboratories, has been appointed superintending scientist of the U. S. Navy Electronics Laboratory, San Diego, California. Mr. Maxfield will be in charge of the Laboratory's scientific and technical research in the fields of radio, radar, and sonar.

A. McGehee Harvey, professor of medicine at Johns Hopkins University School of Medicine, will deliver the annual lecture before the Institute of Medicine of Chicago and the Chicago Society of Internal Medicine on October 22 at the Palmer House. Dr. Harvey will speak on "Some Physiological Experiments of Nature in the Field of Neuromuscular Function; Potassium Deficiency, Potassium Intoxication, Myasthenia Gravis."

William John Miller, professor of geology at the University of California, Los Angeles, since 1924, retired from active teaching on June 30 of this year, thus completing a distinguished career of 47 years as a teacher of geology. Prof. Miller is the author of numerous publications, principally in the field of petrology, and of widely used textbooks in elementary geology.

Bennett Sallman, formerly of the Department of Bacteriology, Ohio State University, has been appointed an instructor in the Department of Bacteriology, Hahnemann Medical College of Philadelphia.

John A. Fleming, of the Carnegie Institution of Washington, has been elected Honorary Fellow of the Geophysical Observatory, University of Alaska, according to word received from S. L. Seaton, Observatory director. Dr. Fleming was cited for his work in the field of terrestrial magnetism and allied investigations in earth physics and for his furtherance of international scientific relations.

Visitors to U. S.

M. Georges Aubert, distinguished soil scientist of the French Ministry of Colonies, is now visiting his colleagues in the United States. He has already completed a considerable tour of this country, after which he consulted with soil scientists of the U. S. Department of Agriculture and the Food and Agriculture Organization of the United Nations in Washington. Before returning to France about November 15 he will lecture at several American universities.

Alex R. Todd, professor of chemistry at Cambridge University, is to be the Nieuwland Lecturer in Chemistry at the University of Notre Dame this fall. Prof. Todd's lectures, on "Vitamins, Coenzymes, and Nucleotides," "The Structure and Synthesis of Nucleotides," and "Phosphorylation and Synthesis of Nucleotides," will be given in the Biology Auditorium at 8:00 P.M. on October 22, 25, and 27.

Karl M. Silberschmidt, chief of the Section of Plant Physiology, Instituto Biológico, São Paulo, Brazil, recently arrived in the United States. During his four-month visit, Dr. Silberschmidt will visit the USDA and other institutions where studies of virus diseases of plants and the physiology of grafted plants are being made.

Max von Laue, director of the Institute for Theoretical Physics, University of Berlin, was awarded the honorary D.Sc. degree at recent University of Chicago convocation ceremonies. The former Nobel Prize winner, cited as "physicist and resolute champion of freedom," was

honored for his discovery and interpretation of the diffraction of X-rays by crystals.

Sir Lawrence Bragg, Cavendish professor of physics at Cambridge University, England, will give a series of 5 lectures at the Mellon Institute, Pittsburgh, Pennsylvania, sponsored by the University of Pittsburgh. The lecture topics will include: "Research Program of the Cavendish Laboratory," November 17; "The Laws of Plastic Flow," November 18; "The X-Ray Structure of Proteins and Other Organic Molecules," November 19; "The Physics of Metals," November 22; and "Recent Developments in the X-Ray Study of Minerals," November 23. Hosts for the various lectures, in addition to the University of Pittsburgh, will include the Pittsburgh Section of the ACS, the X-Ray Society of America, the American Society for Metals, the American Institute of Mining and Metallurgical Engineers, and the American Ceramic Society.

Prior to his visit to Pittsburgh, Sir Lawrence, who will be accompanied by Lady Bragg, will receive the Roebling Medal of the Mineralogical Society of America. The presentation will take place during the Society's 29th annual meeting, November 11–13, at the Hotel Pennsylvania, New York City.

Rudolf Florin, professor of botany and director of Hortus Bergianus, Stockholm, Sweden, has been announced as the holder of the John M. Prather Lectureship in Biology for 1948 by the Department of Biology, Harvard University. Dr. Florin began his series of 7 lectures for those interested in the technical aspects of plant evolution on October 11. Future lectures (October 18, 21, 25, 28, and November 1) will be given at 5:00 P.M. in the Biological Laboratories, 16 Divinity Avenue, Cambridge. A lecture of general interest to the public, "Evolution in Cordaites and Conifers," will be presented on Friday, November 5, at 5:00 P.M. at the Institute of Geographical Exploration, 2 Divinity Avenue, Cambridge.

On September 27–28 Dr. Florin lectured at the University of Illinois, under the auspices of the Department of Botany, on "Paleozoic Cordaites and Conifers" and "Upper Permian and Mesozoic Conifers and Taxads."

Friedrich R. Baltzer, director of the Zoological Institute at Bern University, Switzerland, is a visiting professor in the Department of Zoology at the State University of Iowa for the current academic year. Prof. Baltzer is assuming the duties of **Emil Witschi,** who is on leave of absence for a year and, under a Rockefeller traveling fellowship, is working at the University of Tuebingen in the French Zone of Germany.

Awards

Franklin Medals, highest honors of the Franklin Institute, will be awarded on October 20 to Wendell M. Stanley and Theodore von Karman. Dr. Stanley, head of the Departments of Biochemistry on the Berkeley campus and in the Medical School of the University of California in San Francisco and also director of the University's Virus Laboratory, is cited for "his discovery that a virus can be a nucleoprotein rather than a living organism, thereby establishing a significant turning point in virus research; and also his development of an effective centrifuge-type influenza vaccine by means of which the intensity of future influenza epidemics may be greatly lessened." Dr. von Karman, chairman of the Scientific Advisory Board of the U. S. Air Forces and director of the Guggenheim Aeronautics Laboratory, receives the medal "in recognition of his outstanding engineering and mathematical achievements, particularly those relating to the development of advanced aerodynamic conceptions which have directly influenced the progress of aeronautical design, and for his unusual leadership whereby some measure of his own genius is constantly instilled in those who work with him."

Winners of the Franklin Medal in previous years have included Thomas Edison, Guglielmo Marconi, Neils Bohr, Orville Wright, Albert Einstein, Harlow Shapley, Enrico Fermi, and Sir Robert Robinson.

Colleges and Universities

A conference on statistics applied to research in the social, plant, and animal sciences, held at Auburn, Alabama, September 7–9, was attended by 92 applied scientists and statisticians from 14 states and the District of Columbia. The conference was sponsored by Alabama Polytechnic Institute in cooperation with Iowa State College, the University of North Carolina, Virginia Polytechnic Institute, the U. S. Department of Agriculture, and southeastern Agricultural Experiment Stations. Financial assistance in the form of a grant-in-aid was received from the General Education Board.

Lectures on techniques and designs involved in the application of statistical techniques to research problems in the above-mentioned sciences were followed by brief research reports illustrating the use of the statistical techniques in actual experimental situations. One session was allocated to a discussion of the organization and promotion of statistical activities in colleges and universities, with special reference to regional cooperation in the Southeast.

The lectures in full, accompanied by abstracts of the brief research reports, will be made available in the near future by the Statistical Laboratory of Alabama Polytechnic Institute.

Cornell University's program in cultural anthropology, inaugurated a year ago, is soon to be expanded according to Leonard S. Cottrell, Jr., head of the Department of Sociology and Anthropology and dean of the College of Arts and Sciences. The five-year program, which is designed to survey the impact created by the introduction of Western agricultural, industrial, and scientific methods to primitive societies, has been made possible by a grant of $180,000 from the Carnegie Corporation. During the next four years the program will be divided into two phases—teaching and research. Special research personnel will be trained to study the results of social changes on foreign cultures, and the University will offer new courses, based on the results of field investigations, to foreign and American students planning on overseas careers in the missionary, agricultural, and industrial fields.

Areas currently being investigated include the Far East, India, Latin

merica, and the southwestern United tates. Joining in the research phase the program are R. Lauriston Sharp d Alexander H. Leighton, Cornell ofessors, and three new faculty mem-rs—Allan Holmberg, authority on tin-American cultures who was for-rly associated with the Smithsonian stitution and who has recently been rking in Peru and the Amazon sin; Morris E. Opler, formerly of arvard, whose specialty is Amerin-an and Asiatic cultures; and John Adair, formerly of the University of ew Mexico, who is an expert on the chnologies and handicrafts of the merican Indian.

Supplementary courses are also ing offered by various other depart-nts in the University.

The University of California at Los Angeles is conducting a special urse, set up by the State Depart-nt, for a group of 9 foreign stu-nts (7 from the Philippines, 1 from razil, and 1 from Peru) to advance e study of meteorology in Western emisphere countries and the Pacific d to further the "good neighbor" olicy. The program, administered the U. S. Weather Bureau, is under e direction of Jacob Bjerknes, pro-ssor of meteorology, and Morris eiburger, associate professor of eteorology, is acting as counselor.

Two new courses in the soil me-anics field are being offered by CLA, according to L. M. K. Boelter, an of the College of Engineering. oth courses deal with the principles nd structural applications of soil me-anics. The new program is under e direction of C. Martin Duke, as-tant professor of engineering, D. . Krynine, visiting professor, and eorge Tauxe, lecturer. Prof. Kry-ine, author of several textbooks on il mechanics, has just returned from otterdam, Holland, where he attended e 2nd International Congress on oil Mechanics.

Ohio State University, which was 5 years old on September 17, inaugu-ated a series of anniversary events r a University-wide program, Octo-er 14–15. Five years after it was hristened the "Ohio Agricultural nd Mechanical College" it became hio State University. On the day of its opening in 1873 the entire student body consisted of 15 men and 2 women, and the only college building was what is now University Hall. The University began its 76th year of instruction last month with some 23,000 students, 70 permanent modern buildings, 1,400 acres of campus and farm, and a 400-acre airport. Throughout the 1948–49 academic year the University, in a variety of ways, will celebrate its phenomenal growth.

Industrial Laboratories

Six nuclear physics charts have been prepared by the School Service of the Westinghouse Electric Corporation, Pittsburgh 30, Pennsylvania, for use in school and college classrooms. Depicting the important areas of nuclear physics, the charts, measuring 25″ × 36″, are printed in two colors on heavy stock. The complete set of charts, accompanied by a 32-page book of supplementary information, is priced at $1.00. The first Westinghouse chart portrays 10 basic particles important in nuclear physics; the second explains how nuclei are put together and depicts some 635 isotopes and 75 isomers. Natural and man-made nuclear reactions are shown in the third chart, while the fourth portrays 11 types of apparatus for detecting and inducing nuclear reactions. The fifth chart shows 5 areas for the useful application of atomic energy, while the sixth covers the major theoretical, experimental, and engineering achievements in nuclear physics.

Meetings and Elections

A Conference on Electronic Instrumentation in Nucleonics and Medicine will be held in New York City November 29–December 1, under the joint sponsorship of the Institute of Radio Engineers and the American Institute of Electrical Engineers. Purpose of the conference will be to show the unique problems involved in the utilization of atomic energy and the need for cooperation and understanding between the electronics engineer, the physical scientist, and the medical doctor.

At the Conference the Atomic Energy Commission will present the first comprehensive display of radiation detection instruments essential to the industrial, medical, and biological applications of nuclear energy. The AEC exhibit will include 22 types of basic instruments for radiation detection, together with demonstrations of other instrument techniques.

The California Mosquito Control Association will hold its annual conference jointly with the American Mosquito Control Association at Berkeley and Oakland, California, February 6–9, 1949. The program will cover national, international, and local California problems affecting mosquito control agencies. Following the conference, a field trip will be held (February 10–15) to observe the work and problems of a number of California Mosquito Abatement Districts. Individuals interested in the program are invited to attend. Further information may be obtained by writing to the California Mosquito Control Association, P. O. Box 649, Berkeley 1, California.

The 5th International Congress for Comparative Pathology will be held at Istanbul, May 17–20, 1949, and will be under the chairmanship of Akil Moukhtar Ozden. Papers on problems of medical, veterinary, or plant pathology sciences may be submitted to: Dr. Louis Grollet, Secrétaire Général, Comité International Permanent des Congrès de Pathologie Comparée, 7 rue Gustave Nadaud, Paris, 16e, France.

The Ecological Society of America, at its annual meeting at College Park, Maryland, on September 11, elected the following officers for 1949: president, Z. P. Metcalf, North Carolina State College; vice-president, Charles E. Olmsted, University of Chicago; secretary, William A. Castle, Mary Washington College of the University of Virginia, Fredericksburg; and treasurer, Henry J. Oosting, Duke University.

The Botanical Society of America, Inc., elected the following officers for 1949 at its meetings in Washington, D.C., September 12: president, Dean Ivey F. Lewis, Miller School of Biology, University of Virginia; vice-president, Katherine Esau, University of California; and member of the Edi-

torial Board, Gilbert M. Smith, Stanford University, California. John S. Karling, Purdue University, Turman G. Yuncker, DePauw University, and Ronald Bamford, University of Maryland, continue as secretary, treasurer, and business manager, respectively.

Honorary membership in the American Institute of Chemists will be conferred on Charles Lathrop Parsons at a meeting on October 20 sponsored jointly by the New York Chapter of AIC, the New York Section of ACS, and the American Section of the Society of Chemical Industry. Dr. Parsons is honored for his many and outstanding contributions to the profession of chemist. The dinner meeting will be held in the Downtown Athletic Club, 18 West Street, New York City, at 7:00 P.M. Reservations should be sent in advance to: Dr. Maurice J. Kelley, Nopco Chemical Company, Harrison, New Jersey. Speakers will include Walter J. Murphy, editor of *Chemical and Engineering News;* Donald B. Keyes, of the Heyden Chemical Corporation; and Lawrence H. Flett, Martin Meyer, Clifford F. Rassweiler, and Archie J. Weith, officials of the sponsoring bodies.

Deaths

Samuel E. Sheppard, 66, retired research scientist of the Eastman Kodak Company, died September 29 at his home in Rochester, New York.

Ruth Wheeler, 71, professor emeritus of physiology and nutrition at Vassar College, died September 29 in the Vassar Brothers' Hospital, Poughkeepsie, New York, after a long illness.

Louis F. Ruschhaupt, 69, associate professor emeritus of clinical medicine at Marquette University and staff physician at the County General and Deaconess Hospitals, Milwaukee, Wisconsin, died September 30 after a long illness.

William Roth Work, 67, assistant director of the College of Engineering and Science, Carnegie Institute of Technology, died October 3 in Mercy Hospital, Pittsburgh. He had been associated with the Institute since 1905.

Victor C. Myers, 65, director of the Department of Biochemistry in the School of Medicine at Western Reserve University, died in New York City October 7 while attending the graduate fortnight of the New York Academy of Medicine.

Josephine Owen Cattell, 83, widow of J. McKeen Cattell, died October 10 in Lancaster, Pennsylvania. Mrs. Cattell assisted her husband in the almost 50 years during which he edited *Science*. Following his death in January 1944, she continued, with her son Jaques, to edit the journal until the editorial offices were moved to Washington, D. C., in 1946.

The change in chairmanship of the Research and Development Board, announced last week by President Truman, becomes effective today (October 15). Vannevar Bush, who headed the joint Army-Navy Research and Development Board and, upon unification of the services, the present Board, has resigned and will continue his association with the Carnegie Institution of Washington, which he has headed since 1939. His successor to the post is Karl T. Compton, president of Massachusetts Institute of Technology, who has frequently been called upon to assist the Government on various projects. Among the many governmental tasks which he undertook were the headship of the Office of Field Service of the Office of Scientific Research and Development and the chairmanship of the President's Advisory Commission on Universal Training. He therefore assumes his new duties with a most fitting background for this key civilian position in the National Military Establishment. Upon Dr. Compton's resignation, James R. Killian, Jr., vice-president of MIT since 1945, was elected to the presidency. He is 44 years old, a graduate of MIT, and brings to his new position a wide range of educational and administrative experience.

The American Sociological Society has designated Yale University as the headquarters for its official publication, *The American Sociological Review*. The journal, now in its 13th year, will be under the editorial supervision of Maurice R. Davie, William Graham Sumner professor of sociology at Yale. Prof. Davie will be aided by a group of assistant editors chosen from various institutions. The managing editor is Ernest R. Mowrer, of Northwestern University.

Research Corporation has announced that the services of its present Chicago sales office at 122 South Michigan Avenue will be expanded to facilitate grants-in-aid for the advancement of science in midwestern educational and scientific institutions. Walter E. Thwaite, Jr., formerly an electrical engineer with the Consolidated Edison Company, who joined the staff of the New York headquarters earlier this year, will be in charge of the new phases of operation.

This unusual nonprofit commercial and educational enterprise, was organized in 1912 by Dr. Frederick Gardner Cottrell, a scientist and successful inventor. Endowed by his gifts of valuable patent rights on the Cottrell Process of Electrical Precipitation, it had as its objective the further advancement of science and technology. Dr. Cottrell's plan was to use the proceeds of applied research as grants-in-aid to colleges, universities, and scientific institutions to support research in the physical sciences, mathematics, and engineering.

As the corporation grew, its income was derived largely from the manufacture and sale to industry of the equipment which removed the materials suspended in gases, and this continues today as a major source of its earnings. As time went on, other public-spirited inventors and institutions assigned their inventions to the corporation. Research Corporation now holds inventions or patent rights in 23 commercial fields ranging from aeronautics to vitamins.

The earnings from electrical precipitation and patent management have been distributed by Research

rporation in various ways for the vancement of science. Its largest esent program is the Frederick rdner Cottrell Postwar Program, r which a fund of $2,500,000 was aside for distribution in the 5-year riod from 1946 to 1951. Under his program grants-in-aid to support search are made to educational and ientific organizations after approval s been given by an advisory committee of outstanding scientists. Preference is given to young applicants nd to those with good war records. ositive aid is provided by the Corporation's grants of funds which will llow young scientists to pursue their esired research, thus attracting them to go into, or to return to, teaching nd laboratory jobs in the academic stitutions.

Another phase of the Corporation's ork is the program supported by the Williams-Waterman Fund, derived rom royalties from the patents for he synthesis of vitamin B_1 which Dr. obert R. Williams and his associates ssigned to the Corporation several ears ago. Grants in this case are ade for support of research in combatting dietary deficiency diseases.

Support is also occasionally given to institutions or individuals in special ases for research which does not fit ither of the above programs.

Through the Corporation's Patent Management Department, patent problems are handled for several colleges, niversities, and scientific organizations. Certain inventions made by staff members of these institutions are, by agreement, assigned to the Corporation, which then, at its own expense, attempts to secure patents. When patents are obtained, the Corporation then attempts to get them into commercial use. Income from royalties from these is divided between the inventor, his institution, and Research Corporation in accordance with the agreements between them.

At present some 200 Research Corporation grants amounting to about $900,000 are active in the United States. Fifty-nine of these are placed in the 11 western states, with a value somewhat in excess of $250,000; 82 are placed in the midwestern states, with a value of approximately $335,000.

Through its three offices (New York, Chicago, Los Angeles) the Corporation now hopes to maintain closer relations with scientific and educational institutions throughout the country.

Limited quantities of hydrogen-3 and helium-3, two important new research materials, have been added to the list of isotopes being distributed by the Atomic Energy Commission to scientists and research institutions throughout the country. Hydrogen-3, or tritium, is a radioactive gas with a half-life of about 12 years. As the only radioisotope of hydrogen, it should prove of special value as a "tracer" in medical, biological, and chemical research.

Helium-3, a stable isotope, is only one-millionth as abundant in nature as ordinary helium, which is used in balloons and dirigibles. Now available for laboratory study, it may provide valuable clues to the still largely unknown properties of the helium nucleus. Inquiries regarding the two newly-available materials should be addressed to the Isotopes Division, Atomic Energy Commission, P. O. Box E, Oak Ridge, Tennessee.

The Atomic Energy Commission has also reported that Chile recently became the 22nd foreign nation to qualify to receive radioisotopes for various research projects.

The National Multiple Sclerosis Society has announced the appointment of Cornelius H. Traeger as its medical director. His appointment was made possible by a recent grant of $50,000 from the Milbank Memorial Fund. Dr. Traeger is attending physician and co-chief of the Arthritis Clinic at the Hospital for Special Surgery and consulting attending physician in internal medicine and chief of the Arthritis Clinic at Roosevelt Hospital, New York City.

Formed about two years ago to stimulate interest and finance research in multiple sclerosis and its many allied neurological diseases, the Society to date has financed 7 research projects. Among these are two multiple sclerosis research clinics in Boston, Massachusetts, and Albany, New York. It is planned to finance other worthwhile research projects when adequate funds are available. Dr. Traeger, as medical director, will administer and coordinate medical research in this field.

The National Registry of Rare Chemicals, 35 West 33rd Street, Chicago 16, Illinois, is currently interested in obtaining the following "wanted" chemicals: lecithin phosphatase, *p*-tolylphosphorous dichloride, pyrosulfuryl chloride, 2-fluoroethanol, copper hydride, pelargonin chloride, *l*-borneol, mesobilirubinogen, clupanodonic acid, altrose, tagatose, isofenchyl alcohol, myrtenol, sabinol, verbenol, thujyl alcohol, bufotenidin, 5-(hydroxymethyl)-2-furaldehyde, hyodesoxycholic acid, phosphopyruvic acid, and coproporphyrin.

The 35th edition of "The Naturalists' Directory" has recently been published. This publication contains not only the names, addresses, and special subjects of study of both professional and amateur naturalists of North and South America and some foreign countries but also a list of scientific periodicals and Natural History Museums. Copies may be obtained from the Cassino Press, Salem, Massachusetts. The price is $3.00.

Make Plans for—

American Association of Textile Chemists and Colorists, 27th national convention, October 21–23, Sheraton Bon Air Hotel, Augusta, Georgia.

Symposium on Ultrasonics and Macromolecules, October 23, Institute of Polymer Research, Polytechnic Institute of Brooklyn.

National Metal Congress of American society for Metals, October 25–29, Benjamin Franklin Hotel, Philadelphia, Pennsylvania.

Gas Discharge Conference, October 27–29, Brookhaven National Laboratory, Upton, New York.

American Mathematical Society, October 30, New York City.

National Committee for Mental Hygiene, 39th annual meeting, November 3–4, Hotel Pennsylvania, New York City.

Comments and Communications

Denaturation of Urease Without Inactivation

It appears to be generally believed that the denaturation of an enzyme always coincides with its inactivation. This has been found to be true even when the enzyme could be partly reactivated later. It appeared of interest, therefore, to bring to attention one of our early papers (J. B. Sumner and V. A. Graham. *Proc. Soc. exp. Biol. Med.*, 1925, 22, 504), in which we describe the preparation of an insoluble, although still highly active, urease. This material was obtained by allowing partially purified urease in 30% alcohol and sodium chloride to stand at room temperature for 1 or 2 days.

Recently I have prepared insoluble urease from once-recrystallized urease. The material is quite insoluble in water or dilute phosphate buffer and is highly active, although considerably less active than soluble urease. It gives a strong nitroprusside test for sulfhydryl groups and is much more resistant to digestion by commercial trypsin at pH 7.0 and 30° C than the same material after boiling. It is evident, therefore, that the reactions which occur when urease is denatured in this manner do not destroy all of the active groups.

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Human Saliva as a Germination Inhibitor

The inhibiting effect of saliva on the germination of wheat seeds, of which D. Yardeni (*Science*, July 16, pp. 62–63) presents a series of interesting examples, is attributed by her to the occurrence of bacteriostatic substances in human saliva.

According to the results of Yardeni's experiments, the inhibiting factor is heat resistant, nonvolatile, removable by dialysis, and affects the growth of the radicles more than that of the coleoptile. One may well wonder whether all these effects could not be easily explained by the eventual growth-hormone content of saliva, which is not even mentioned. As early as 1925 E. Seubert (*Z. Bot.*, 1925, 17, 49) produced curvatures of *Avena* coleoptile by unilateral application of saliva. A. J. Haagen-Smit, in his general review on this subject (*Ergebn. Vitamin Hormonforsch.*, 1944, 2, 355), refers to that paper. H. Lane (*Amer. J. Bot.*, 1936, 23, 532–535), K. V. Thimann (*Amer. J. Bot.*, 1936, 23, 561–569), Th. Solacolu and D. Constantinesco (*C. R. Soc. Biol. Paris*, 1937, 124, 492–494), and many other authors have demonstrated the inhibiting effect of growth hormones on the root growth of germinating seeds. That urine and other animal body liquids contain growth hormones has been proved principally by F. Kögl, A. J. Haagen-Smit, and H. Erxleben (*Z. phys. Chemie*, 1933, 220, 137–161) and confirmed by many other authors—for instance, F. Ubatuba (*Rev. Bras. Biol.*, 1946, 6, 23–62). Thus, it seems to us that the possibility of growth hormones, present in human saliva, being responsible for its germination-inhibiting effect should be taken into consideration.

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Absence of Protective Action of Rutin in Anaphylactic and Histamine Shock

In a recent study of the influence of rutin on anaphylactic and histamine shock, Raiman, Later, and Nechele (*Science*, October 17, 1947, p. 368) reported that significant protection was obtained when sensitized guinea pigs were pretreated with 1 or 2 mg (total dose) of rutin 30–45 min prior to shocking with the sensitizing antigen, but they found no protection by rutin against histamine shock. Wilson, Mortarotti, and DeEds (*J. Pharm. exp. Therap.*, 1947, 90, 120) earlier had reported a significant reduction in mortality from histamine shock using a higher dose of rutin, but briefly mentioned their inability to demonstrate any protection against anaphylactic shock.

Because of these conflicting results, we repeated such experiments on several series of animals. All rutin injections were intraperitoneal, and all injections of antigen or histamine were via the dorsal penile vein, a route which eliminates the complications of cardiac damage or false shock symptoms which may attend piercing the pleural cavity.

The first series of guinea pigs, sensitized with horse serum, was divided; one group was carried through procedures duplicating in all details those of Raiman, *et al.* except for the route of intravenous injections. The other group received larger doses of rutin, and in some the time interval was shortened. Table 1A shows that the preponderant results were severe or fatal anaphylaxis, with only 3 of 25 animals showing no symptoms. More than half of the remainder succumbed, with typical post-mortem findings of anaphylactic shock. The low incidence of mortality in the control group (50%) was regarded as unsatisfactory, and another series was started.

In the second series, the guinea pigs were sensitized by a multiple-site intramuscular injection of 0.4 cc of a suspension of Freund adjuvant in fresh egg white (J. Freund and K. McDermott. *Proc. Soc. exp. Biol. Med.*, 1942, 49, 548). This technique has been found by previous experience to produce a high degree of sensitization in all animals. The shocking dose of 0.4 cc of a 1:8 dilution of fresh egg white, given 21–26 days later, has been found to be the LD_{100} dose for our animals, in agreement with the data of Marcus (*Proc. Soc. exp. Biol. Med.*, 1947, 66, 181). In this series, all 20 control animals died, and the same dose was used for the pretreated animals. The amount of rutin was increased to 10 mg (total dose) and was given as a 10% solution of the methyl-glucamine salt, having a pH of approximately 8.0. Methyl glucamine *per se* has been found to be pharmacologically inert, while rutin thus prepared was shown

be active when tested by the capillary permeability thod reported by Ambrose and DeEds (*J. Pharm. exp. erap.*, 1947, **90**, 359). The variable introduced was the e interval between the rutin injection and the shocking se of antigen. Table 1B shows that again no significant otection was shown in the 13 animals tested.

The third series of animals was pretreated with 10 mg rutin (methyl-glucamine salt) before shocking with tamine. In these, the histamine dose was 0.4 mg/kg base), which in our experience agrees with reports other investigators as the LD_{100} dose. The interval tween rutin and histamine was varied between 5 and min. In no instance was protection demonstrable, as own in Table 1C.

TABLE 1

INFLUENCE OF RUTIN PRETREATMENT IN ANAPHYLACTIC AND HISTAMINE SHOCK

Rutin (mg, IP)	Interval before shocking (min)	Total animals shocked	Shock, absent	Shock, non-fatal	Shock, fatal
None	Control	8	0	4	4
1	30–35	4	1	1	2
2	45	7	0	5	2
4	45	7	2	2	3
10	45	3	0	1	2
20	45	1	0	0	1
5	30	1	0	0	1
10	30	1	0	0	1
None	Control	20	0	0	20
10	10	2	0	0	2
10	15	4	0	2	2
10	25	3	0	0	3
10	30	1	0	0	1
10	35	2	0	1	1
10	40	1	0	0	1
None	Control	10	0	0	10
10	5	2	0	0	2
10	10	3	0	0	3
10	15	2	0	0	2
10	45	2	0	0	2

A. Horse-serum sensitized guinea pigs, intravenous horse-rum shocking dose.

B. Egg white-Freund adjuvant sensitized guinea pigs, in-venous dilute egg white LD_{100} shocking dose.

C. Nonsensitized guinea pigs, intravenous histamine LD_{100} ocking dose.

It might be suggested that some variability in the de-ree of sensitivity of the guinea pigs might account for e apparent slight protection following rutin pretreat-nt. This conclusion could have been drawn from our wn first series, had not chance introduced the low mor-lity incidence in the controls as well. When the ex-riments were repeated with highly sensitized animals, sults were more definitive. The relatively small num-r of animals makes statistical evaluation difficult. owever, application of the Chi-square method gives a value between 0.02 and 0.05, which is considered only the borderline of significance. With respect to the sparity of results with histamine shock, it should be inted out that we used the LD_{100} dose of histamine, while Wilson, *et al.* used the LD_{50} amount. We have frequently observed that minimal doses of an anti-histaminic, incapable of preventing ultimate death, may nevertheless delay for some time the fatal outcome following a lethal dose of histamine, whereas in our rutin series, no prolongation of life was noted, all animals dying within 5 min. Our agreement with Raiman, *et al.* would indicate that rutin protection is insignificant when the higher amount of histamine is used.

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Red Skin Color of Bliss Triumph Potatoes Increased by the Use of Synthetic Plant Hormones[1]

During the course of experiments with synthetic plant hormones on potatoes an increase in the red skin color of the variety Bliss Triumph was noted. Sodium and ammonium salts of 2,4-D and the butyl ester were applied to the soil as a side dressing when tubers were approximately one-third grown. Rates of 20 lbs, 200 lbs, and 400 lbs/acre of the acid equivalents were used. No apparent injury to the plants or reduction in yield was noted with the 20 lbs/acre, but serious plant injury and yield reduction occurred where 200 and 400 lbs/acre were applied. All rates showed an increase in the red skin color of Bliss Triumph tubers grown in sandy soil. No change in flesh color or flavor of the treated tubers was noted. Since a deep red color in Bliss Triumph potatoes is a highly desired market character, this finding may prove of considerable economic importance.

Further tests are in progress to determine minimum amounts required for increasing red color of potatoes.

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L. A. SCHAAL
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On Olfaction and Infrared Radiation Theories

The work of Beck and Miles on odor experiments with bees (see Abstract of paper presented before National Academy of Sciences, *Science*, November 28, 1947, p. 512), recently brought to some popular attention, has revived interest in theories concerning the possibility of an olfactory sense mechanism in which radiation of wave lengths characteristic of molecular vibration frequencies (infrared or Raman spectra) plays a part. There is a considerable body of discussion in the literature about such theories (cf. R. W. Moncrieff. *The chemical senses.* New York: John Wiley, 1946).

We should like to emphasize several points having a bearing on these theories which we believe require more

[1] Contribution from the Department of Botany and Plant Pathology, Colorado Agricultural Experiment Station, Fort Collins, in cooperation with the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, USDA. Published with the approval of the director of the Colorado Agricultural Experiment Station as Scientific Journal Series Article No. 284.

scrutiny before any noteworthy importance can be attributed to them. One such point is concerned with the properties of isotopic molecules. For example, we have had occasion to prepare the deuteroxyl counterpart of *n*-butyl alcohol. The deuterated compound has indistinguishably the same odor as the original alcohol, although, of course, the infrared spectra differ to some extent as a result of the substitution. The spectra of odorless ordinary water and odorless heavy water, it is well known, are quite dissimilar. We suspect that no evidence has been found, or is likely to be found, that the odor of any isotopically substituted molecule differs from that of its ordinary counterpart.

On the other hand, there is some evidence that *d*- and *l*- forms of a few optical isomers have different odors (J. von Braun and W. Kaiser. *Ber.*, 1923, 56B, 2268; J. von Braun and E. Anton. *Ber.*, 1927, 60B, 2438). Here the infrared spectra (or Raman) are identical (barring the effect of polarized radiation, presumably not to be considered in the question at issue). Clearly, a difference in odor between optical isomers must be explained by some effect other than infrared absorption.

These two points would seem to offer an insoluble contradiction to any sort of infrared radiation theories concerning (at least) the human olfactory sense.

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The Curare-like Action of Thiamine

Demole (*Kongressber. XVI, Int. physiol. Kongr.*, 1938, 19) and Pick and Unna (*J. Pharm. exp. Therap.*, 1945, 83, 59) mention the curare-like action of thiamine in frogs and rats. The present preliminary paper presents additional evidence for the curare-like action of thiamine.

Thiamine hydrochloride,[1] by rapid intravenous injection of 50 mg/kg or more into dogs, causes respiratory paralysis, hypotension, bradycardia, and vasodilation, all of which eventually disappear if artificial respiration is maintained (J. A. Smith, *et al. Fed. Proc.*, 1947, 6, 204; 1948, 7, 116; *J. Pharm. exp. Therap.*, 1948, 93, 294).

Fig. 1 shows that D-tubocurarine,[1] in doses of 1–2 units/kg, produces effects closely resembling those of thiamine (150 mg/kg). The vasomotor action of D-tubocurarine, however, has not yet been investigated fully. The figure also shows the results of experiments in which the sciatic nerve was isolated and stimulated and the resulting contraction of the toe recorded. It is seen that both D-tubocurarine and thiamine stop the contractions resulting from stimulation of the nerve. (The muscle was still responsive to direct stimulation.) These results are typical of substances having curare-like action.

Intocostrin[1] (2 units/cc) and thiamine hydrochloride (5% solution) were injected into the ventral lymph sac of frogs. The sciatic nerve and gastrocnemius muscle were exposed. It was found that intocostrin (80 units/kg) and thiamine (2,000 mg/kg) prevent the contraction of the muscle when the nerve is stimulated without decreasing the contraction on direct stimulation.

Thus, except for the dosage, the effects produced by curare preparations are like those produced by thiamine under the conditions of these experiments.

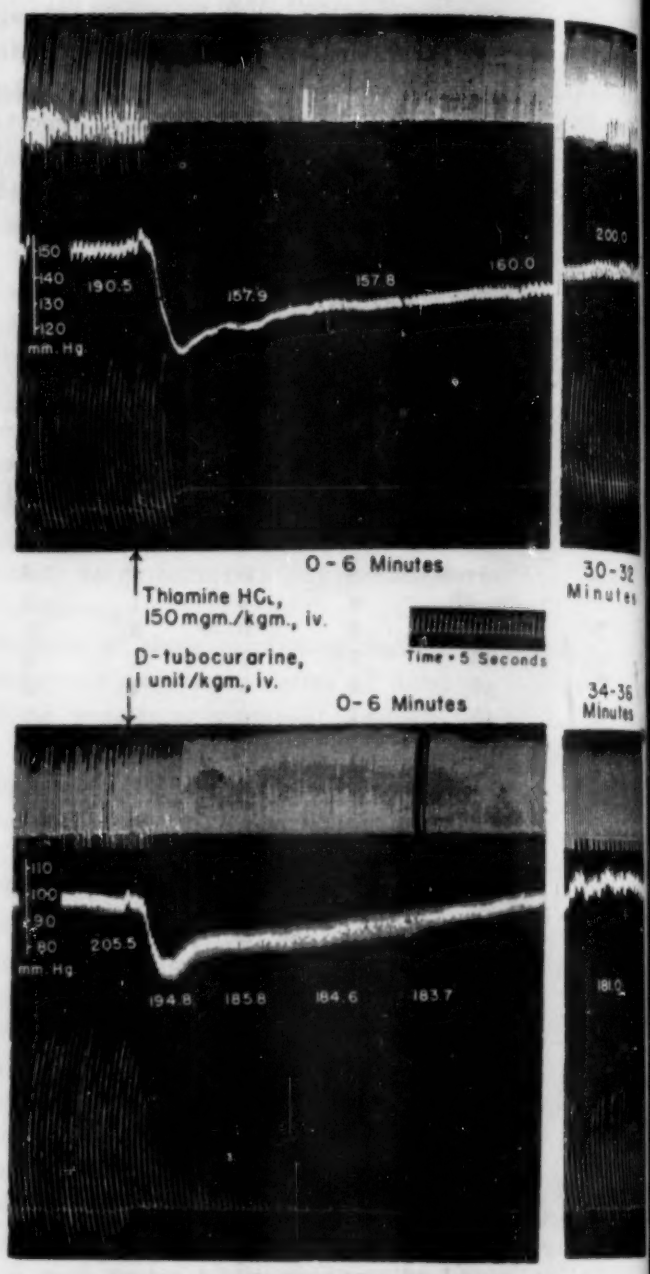

FIG. 1. Effects of intravenous injection of thiamine hydrochloride and D-tubocurarine into dog. Upper recording—respiration. Natural respiration is superimposed on artificial respiration. Middle recording—blood pressure. The heart rate is shown by the numbers along the blood-pressure curve. Lower recording—muscle response recorded from the toe when the sciatic nerve was stimulated at 5-sec intervals throughout the experiment.

The curare-like action of thiamine probably explains many of the results previously obtained by us with thiamine hydrochloride (*J. Pharm. exp. Therap.*, 1948, 93, 294) and by Haley with other thiamine salts (*Proc. Soc. exp. Biol. Med.*, 1948, 68, 153).

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[1] Thiamine hydrochloride was supplied to us by the Upjohn Company, Kalamazoo, Michigan; D-tubocurarine and intocostrin, by E. R. Squibb and Sons, New York.

TECHNICAL PAPERS

ody Temperature, Blood Pressure, d Hypothalamus[1]

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n the course of studies on the regulation of normal od pressure, data were obtained which suggested a eral relationship between body temperature and blood ssure. These studies were then extended to include mmals, birds, reptiles, and amphibians. It soon apred that we were dealing with a more general prinle involving adjustments of the internal environment temperature changes, and this led to a consideration of phylogenetic development of temperature regulation. A comparison of data on the blood pressure of normal ting animals led us to give particular attention to the rage diastolic pressure of unanesthetized mammals inding dog (*2*, *10*), rabbit (*5*), man, and rat which is out 80 mm Hg. These mammals have normal body mperatures approximating 38° C. The chicken (*12*), key, and duck (unpublished data) have diastolic pres- es of 120–140 mm Hg and correspondingly higher body mperatures (about 42° C). These average normal lues are represented by the circles in Fig. 1.

These findings made it appear worth while to determine the blood pressure would change as the body tempera- e was changed. In the turtle the blood pressure was nd to rise with increasing body temperature and to ll as the animal was cooled (*8*). For example, the ssure averaged 21/16 mm Hg at 10° C, and at 37° C reached a maximum level of 39/33 mm Hg (Fig. 1). milar data, not yet published, were obtained with the g. The same relationship was elicited in mammals d birds. Cooling produced a progressive fall in blood ssure in chickens (*12*), dogs, and rabbits (unpublished ata), and rewarming resulted in an equal rise in pressure *ari passu* with the body temperature (Fig. 1).

These relationships hold only within a given tempera- re range for each species. Warming of the mammal or ird above its normal temperature resulted in no consis- nt changes in pressure. At about 3° C above these nor- al temperatures a critical level was reached, and the lood pressure fell rapidly, leading to death. Critical ermal levels were observed in the frog at about 25° C d in the turtle at about 37° C (*8*).

At this point our interest in the mechanism of the mperature-pressure relationship was aroused. Autono- ous changes in the reactivity of the blood vessels could t account for the relationship. Thus, no significant fferences were observed in the pressor response to epinephrine or the depressor effects of acetylcholine in either the hypothermic or hyperthermic chicken (*9*, *12*), rabbit, or the turtle. Small variations were seen in the

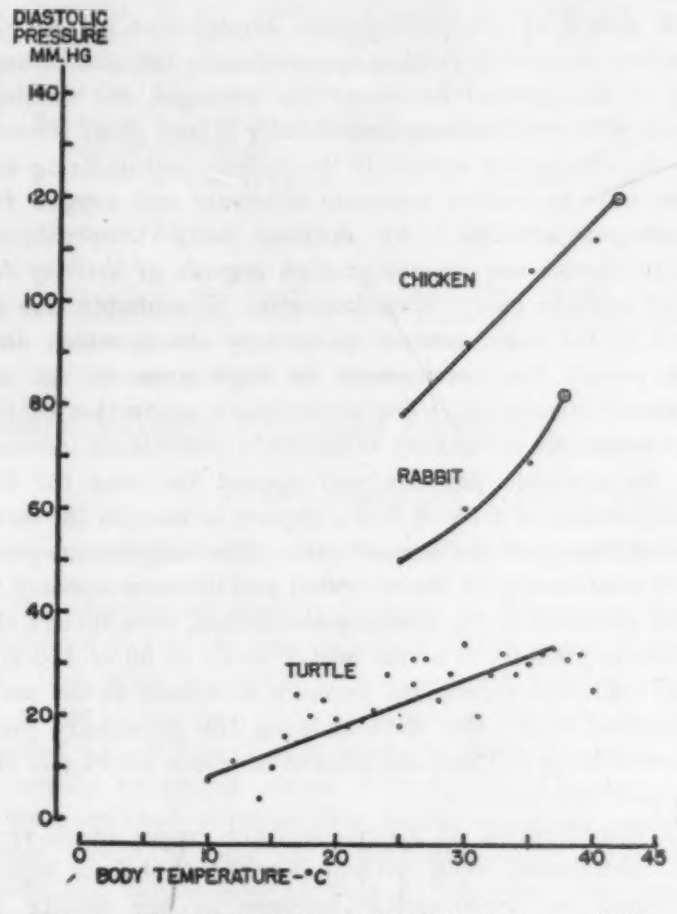

FIG. 1. Body temperature–arterial pressure relationship.

concentration of the plasma protein and in the blood volume. Circulation time, cardiac output, and heart rate were also found to be direct functions of the body temperature. Cooling of the chicken (*9*) and the dog (unpublished data) resulted in a slowing of the circulation and a decreased cardiac output, while rewarming resulted in a speeding up of the circulation. However, the fact that the circulation rate continued to increase in severe hyperthermia even after the blood pressure had begun to fall indicated that the blood pressure changes did not depend solely on these factors.

We then investigated the role of the central nervous system. Destruction of the brain or section of the cervical spinal cord eliminated the effect of body temperature on the blood pressure (*6*). In order to establish whether the temperature-pressure mechanism operated through receptors in the periphery, we tested the effect of thermal stimulation of the brain. This was accomplished by the insertion into the brain of a silver wire connected to a water reservoir. Warming the brain caused an immediate rise in arterial pressure, while cooling caused an immediate fall (*11*). Slight thermal differences caused consistent covariance when the wire was in or near the hypo-

[1] Aided by a grant from the Department of the Army to the Michael Reese Hospital.

thalamus, but not when it was in the medulla or in the olfactory or optic lobes. To our knowledge this is the first direct evidence for a temperature-sensitive center in the brain of a cold-blooded animal.

Changes were found not only in the circulation but also in the concentration of certain elements in the blood. Thus, cooling of the chicken was followed by a fall in blood sugar, and rewarming, by a return to normal levels (*7*).

A review of the phylogenetic development of the circulation suggested further approaches to the understanding of the general problem. For example, the development of homeothermism undoubtedly placed great stresses on the circulatory system if the rapidly metabolizing tissues were to receive adequate substrate and oxygen for prolonged activity. At optimal body temperatures, poikilotherms are capable of high degrees of activity for brief periods only. This limitation is probably due in part to the low-resistance pulmonary shunt, which does not permit the development of high pressures in the systemic circulation. The evolutionary separation of the low-resistance pulmonary shunt made possible an increase in the systemic pressure and opened the way for the maintenance of a blood flow adequate to nourish the more active tissues of the homeotherm. The temperature-pressure relationship of the ancestral poikilotherm appears to have persisted in the systemic circulation, even though the diastolic pressure is at the higher levels of 80 or 120 mm Hg, while the pulmonary pressure continues at the more primitive level. Our data showing the pulmonary pressures of dogs (*3*) and chickens to be about 25/10 mm Hg support this concept.

A consideration of the temperature ranges which various vertebrates could withstand without obvious injury indicates an evolutionary increase in the ability to tolerate rapid changes in body temperature, which has had important bearing on the development of warm-blooded animals (Fig. 2). For example, for fish living in large bodies of water, the ability to adjust to rapid temperature changes is not essential for survival, since such changes do not occur. However, shallow-water fish may be exposed to variations of several degrees during the course of the day and hence must be able to withstand such changes.

This process continued with the evolution of the air-breathing vertebrates. When the fish left the water and lived on the land as amphibians, they were exposed to far greater diurnal temperature changes than occurred under cover of the high specific heat of the protecting water. Within the next 60,000,000 years some amphibians had developed the capacity to tolerate still greater diurnal changes in temperature and were able to leave the water completely, giving rise to the reptiles.

These diurnal environmental temperature changes play an important role in the normal cycles of activity of the terrestrial poikilotherm. It is apparent that early in the morning in temperate climates poikilotherms living on the land have a low body temperature and are at low metabolic and activity levels. Absorption of solar energy during the day raises the body temperature and increases the metabolic activity, as indicated by oxygen consumption, general activity, respiratory and heart rates, blood pressure, and circulation rate. Optimal body temperatures may be regulated by locomotor responses. During

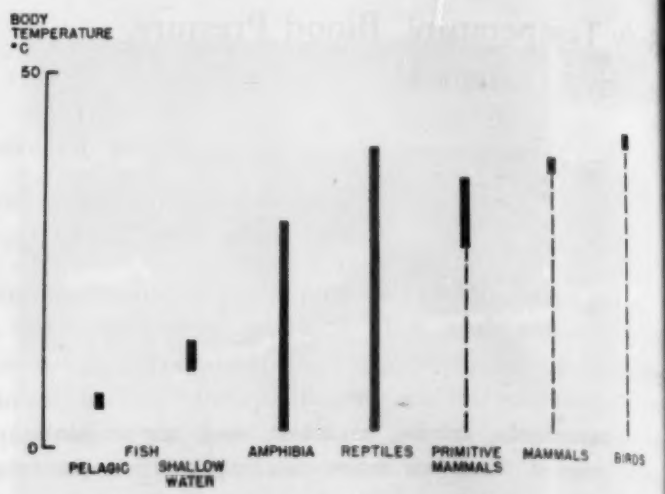

FIG. 2. A schematic representation of the diurnal variations in body temperature of vertebrates. Heavy lines suggest the variations in body temperature which may occur during a single day. Dotted lines indicate the potential temperature changes normally prevented by temperature-regulating mechanisms.

this period the nutritive, defensive, and reproductive activities which result in individual and species survival are performed. With evening, the poikilotherm loses heat. This leads to a decline in metabolism, activity, circulation rate, and blood pressure, and the animal may fall into a sleep-like torpor. Each day this cycle of warming and activity, cooling and torpidity, is repeated.

In the Triassic period, the theriodont reptiles, which may already have developed the ability to withstand large temperature changes, gave rise to the early mammals. Thirty million years later, in the Jurassic, another group of reptiles, which may have increased their diurnal temperature range still more, gave rise to the ancestors of the modern birds. Thus, the birds may have adjusted their normal body temperature at their optimal level, higher than that of the earlier evolving mammals. Hair or feathers conserved the warmth gained during the day and kept metabolism relatively high during the early night, compared with the cold, torpid poikilotherms. Further evolution brought the development of heat-conservation mechanisms, such as the pilomotor function and the ability to shift blood from the skin, and thermogenic mechanisms evolved which came into play whenever the body temperature began to fall. When the increasing efficiency of heat conservation and thermogenic mechanisms brought the danger of raising the body temperature above the critical level, heat-loss mechanisms began to have survival value. Similar patterns of development of temperature regulation have been suggested by observations on newborn mammals and birds (*4*) which show that, ontogenetically, heat-conservation mechanisms precede thermolytic mechanisms.

Our discovery of the thermal sensitivity of the hypothalamic region in the cold-blooded animal (*11*) made it possible to correlate our work with a number of hitherto

uncorrelated data. This miniscule portion of the brain has been deluged with responsibility for a large number of apparently unrelated functions (*1*); it has been considered the head-ganglion of the autonomic nervous system, responsible for the regulation of body temperature, blood pressure, respiration, appetite, the diurnal rhythm of sleep and wakefulness, the sexual cycle, and the control of the metabolism of sugar, fat, and water. On the basis of the approach presented above, these functions may be considered as parts of an integrative mechanism. Thus, it is apparent that as the neo-amphibian leaves the water and is exposed to large changes in body temperature, immense variations may occur in the metabolic rates of the numerous tissue and organ systems which might lead to maladjustments of the internal economy. An internal environment optimal for 5° C might be wholly unsuitable for survival at 30° C. Since temperature variations of such magnitude may occur rapidly in terrestrial poikilotherms under natural conditions, the development of a coordination center for metabolic adjustment would provide survival value for its possessor. In such poikilotherms this center would be charged, not with the maintenance of the constancy of the internal environment (homeostasis) but with the coordination of changes in the internal environment (homeodynamics) to meet the demands placed by other changes such as temperature. Such a rapidly reacting center for homeodynamic control appears to have been developed in the hypothalamus. The rich vascularity of this organ makes it eminently suitable for such a role.

Our studies suggest that some homeodynamic regulations depend upon the independent variable, the temperature of the temperature-sensitive center in the brain. In the course of time, heat conservation, thermogenic and thermolytic mechanisms apparently were laid down proximal to the homeodynamic center, introducing a new factor: the relative constancy of body temperature. Under these conditions, the homeodynamic adjustments became minimal during the diurnal cycle, although the adjusting mechanisms persisted. Patterns of behavior of warm-blooded animals suggest retentions of some elements of the diurnal cycle of the poikilotherms, such as the diurnal variations in body temperatures and the period of torpor (sleep). Phenological functions, such as the reproductive cycle, which previously depended in part upon the diurnal variations and their annual precession, may escape and establish a reproductive calendar independent of the solar year.

Although begun as an analysis of a relationship between body temperatures and blood pressure, our studies have not only indicated that the relationship depends on the central nervous system, but have suggested that it may be but a portion of a more general integration. Some of these integrating mechanisms may be approached experimentally in the poikilotherm by producing variations in body temperature. In homeotherms, the superimposition of a relatively constant body temperature serves to mask the basic integrative mechanisms, but induced hypothermia causes the animal to revert to a more primitive condition and thus exposes the homeodynamic apparatus to experimental analysis.

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Indole-3-Acetic Acid and Flowering

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The experiments described briefly in this paper were conducted to test the assumption by Thurlow and Bonner (*2*) that, since a marked decrease in the auxin content of plants at the time of their flowering has been demonstrated (*1*), it might be possible to delay flowering by externally supplying plants with auxin. Thurlow and Bonner sprayed soybean and *Xanthium* plants, grown under flower-inducing short photoperiods, with indole acetic and naphthalene acetic acid solutions (500 ppm) and observed inhibition of production of flower primordia, a result which lends support to Bonner's assumption. Since the leaves of the treated plants showed pronounced epinasty and other growth deformities and since the inhibitory effect of the spray might thus be an indirect leaf-injury effect, it was thought desirable to treat plants with indole acetic acid by immersing their roots or the cut ends of their stems in water solutions of this auxin in order to avoid direct auxin contact with leaves.

In the first set of experiments the roots of petunia plants (var. Topaz Queen) which were 7 weeks old and which were approaching flowering time, though still without flower primordia, were immersed for 24 hrs in water solutions of indole-3-acetic acid (200 ppm). The plants were then potted in rich loam soil. The first visible flower buds appeared in these treated plants 23 days after the first flower buds were visible on the control plants, roots of which were treated for 24 hrs with water. Thus, the indole acetic acid treatment distinctly delayed flowering, a result which agrees with that of Thurlow and Bonner. No signs of leaf epinasty or other types of deformity appeared in the leaves of the treated plants, nor was there any apparent difference in root development between treated plants and controls.

This experiment was repeated on Lincoln soybeans,

with a similar result. The treated plants produced their first flower buds 12 days after the controls.

A second set of experiments was devised to determine whether or not indole acetic acid solutions might inhibit the development of already-formed flower buds into fully opened flowers. Young inflorescences bearing flower buds in various stages of development but no open flowers were cut from plants of stocks, snapdragons (Rose Queen), annual larkspur (Blue Bell), blue salvia, and iris (Sierra Blue), and were immediately placed with their cut ends in water solutions of indole acetic acid (25, 50, 100, 150 ppm) for 24 hrs; following this treatment, the inflorescences were placed in containers with the cut ends in tap water. On successive days, counts were made of the numbers of buds which developed into fully opened flowers. The results indicate that, in all treated plants, the indole acetic treatment retarded the development of flower buds into open flowers, as compared with that of untreated controls. In iris, the growth of flower buds was completely inhibited; all buds of the controls opened. The retarding effect of the auxin solutions upon bud development was more pronounced at the higher concentrations used; also, at the higher concentrations, some signs of leaf epinasty and of abnormal stem twisting were observed. These growth abnormalties were less pronounced than those of leaves directly treated with auxin sprays.

Although these experiments differed somewhat from those of Thurlow and Bonner, the results are similar to theirs. It cannot be assumed that the results of such experiments demonstrate a causal relationship between auxin content of a plant and its flowering, but these preliminary tests may open a way to more precise approaches to the suggested relationship.

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Observation on the Mechanism of Action of Dicoumarol

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The mechanism of the action of dicoumarol is obscure, and many clinicians hesitate to use it for this reason. Quick (*5*) presents evidence that the prothrombin time, as measured by the one-stage method, may be prolonged by diminution of prothrombin component A, component B, or a labile factor. According to Quick, component B is reduced in dicoumarol administration.

Loomis and Seegers (*2*) consider prothrombin to be a unitary principle and receive support from Munro and Munro (*3*), who have demonstrated that A and B cannot be regarded as separate components.

Accepting the hypothesis of a unitary principle, the clotting time has been studied when dicoumarol plasma is mixed with whole normal plasma, Seitz-filtered plasma, defibrinated plasma, and serum.

Experiment 1—Mixture of Dicoumarol Plasma and Normal Plasma

Dicoumarol plasma (14% prothrombin)		Dilution of normal plasma				
Mixture		80%	50%	25%	12.5%	6.25%
Prothrombin percentage	Observed %	50	42	38	34	22
	Arithmetic avg. %	47	32	19	13	10

Experiment 2—Dicoumarol Plasma and Seitz-filtered Normal Plasma

Dicoumarol plasma (5% prothrombin)		Dilution of Seitz-filtered plasma (free from prothrombin)				
Mixture		80%	50%	25%	12.5%	6.25%
Prothrombin percentage	Observed %	3	3	3	2	3
	Arithmetic avg. %	3	3	3	3	3

Experiment 3—Dicoumarol Plasma and Defibrinated Normal Plasma

Dicoumarol plasma (25% prothrombin)		Dilution of defibrinated plasma* (100% prothrombin)			
Mixture		50%	25%	12.5%	6.25%
Prothrombin percentage	Observed %	30	24	20	17
	Arithmetic avg. %	37	25	18	13

* Plasma was defibrinated by the addition of 1/10 volume of Parke Davis & Co. Thrombin Topical: One ampoule was made up to 200 ml with 0.15 N sodium chloride. The excess thrombin was inactivated by keeping the plasma at 37° for 1 hr.

Experiment 4—Dicoumarol Plasma and Normal Serum

Dicoumarol plasma (24% prothrombin)		Dilution of 24-hr serum* (trace of prothrombin)			
Mixture		50%	25%	12.5%	6.25%
Prothrombin percentage	Observed %	44	38	32	24
	Arithmetic avg. %	12	12	12	12

* The serum was allowed to stand for 24 hrs. It contained a trace of prothrombin by the one-stage method, but no thrombin.

Dilutions of normal were made with 0.9% sodium chloride; 0.1 ml of dicoumarol plasma and 0.1 ml of diluted normal plasma were added to 0.2 ml of thromboplastin (Difco) and the mixture activated by 0.2 ml of 0.025 M calcium chloride solution. The clotting time was measured at 37° C and the corresponding prothrombin percentage read from a dilution curve. This value was compared with the arithmetic average of the prothrombin concentrations of the dicoumarol plasma and the normal plasma, measured separately.

The recorded observations are ones selected at random om experiments using several different dicoumarol asmas.

When normal plasma is added to dicoumarol plasma, e prothrombin content of the mixture is increased more an can be explained by the simple mixing of pro-rombin solutions of different concentrations. Conley d Morse (*1*) recognized this when they added in-agulable plasma from a dog receiving large quantities dicoumarol to normal human plasma.

This observation could be explained by the presence of prothrombin accelerator in normal plasma which is ficient in dicoumarol plasma. When observed values r the mixture are plotted against arithmetically ex-cted values, a logarithmic curve is obtained.

The accelerator is removed from the plasma by Seitz tration. This distinguishes it from Owren's (*4*) factor which passes through Seitz filters.

The accelerator disappears when normal plasma is eated with thrombin. It is present, however, in serum.

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solation of Pure Vitamin A_2[1]

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Since the discovery in 1937 of vitamin A_2 (*1, 4*), a ompound related to vitamin A and found chiefly in the ivers of certain species of fresh-water fish, there has een much disagreement among various investigators with espect to its structure and biological activity. Much of his dissension arises from the lack of criteria by which to easure vitamin A_2. The purpose of this paper is to tablish some of these criteria by reporting the isola-ion of pure (though nonerystalline) vitamin A_2 alcohol hrough a crystalline ester.

During the fall of 1946, 150 lbs of "pike" livers ere collected from local fish markets. Because the rigin of the livers could not be definitely assured, each ver was examined under an ultraviolet lamp. Livers owing the brilliant yellow fluorescence of vitamin A_1 ere discarded, while those showing the characteristic rownish-orange fluorescence of vitamin A_2 were pooled, round, and extracted with ethyl ether. Slightly over kg of rather low-potency oil was obtained.

In the following brief description of the concentration f vitamin A_2 from this oil, the symbol E stands for E (1%, 1 cm) at 351 mμ, the main ultraviolet absorption maximum of vitamin A_2.

The original oil (E = 4.0) was distilled in a centrifugal molecular still to give a distillate containing the natural vitamin A_2 esters (E = 24.2). Redistillation further increased the potency (E = 65.0), and the triglycerides were removed by saponification (E = 182). Sterols were crystallized out in acetone at −30° C (E = 260) and the residue chromatographed on sodium aluminum silicate (E = 482). The chromatographed concentrate was distilled in a high-vacuum pot still (E = 848) and desterolated again in ethyl formate at −30° C (E = 862). The concentrate was then chromatographed on magnesium oxide (E = 1,030), on zinc carbonate (E = 1,250), again on zinc carbonate (E = 1,320), and a third time on zinc carbonate (E = 1,350). The extinction could not be increased by further chromatography. This is about the same extinction as that obtained by Karrer and Bretscher (*8*) in their investigations of vitamin A_2. However, other data reported by these workers (main ultraviolet absorption maximum at 345 mμ instead of 351 mμ, along with relatively low extinctions for the subsidiary band at 287 mμ and for the $SbCl_3$ blue product at 695 mμ) indicate that their preparation probably contained appreciable quantities of vitamin A_1.

Since attempts to crystallize this material from various solvents (methyl alcohol, methyl formate, ethyl formate, Skellysolve "F") were unsuccessful, 1.25 gm of the concentrate was esterified with 1.4 gm of phenylazobenzoyl chloride by allowing it to stand for 4 hrs at room temperature in 20 ml of methylene chloride containing 2 ml of pyridine.

The vitamin A_2 phenylazobenzoate thus obtained was chromatographed twice on zinc carbonate. The ester formed a weakly adsorbed reddish-orange band which was eluted from the column with petroleum ether (Skellysolve "F"). The ester concentrate (1.9 gm) was allowed to stand in 10 ml of Skellysolve "F" at −30° C. After 3 days, crystals formed in hemispherical orange rosettes on the walls of the vessel. Three recrystallizations gave a final yield of 329 mg of tiny orange prisms which melted at 76°–77° C (Fisher-Johns apparatus, uncorr.). *Analysis*: required for $C_{33}H_{38}N_2O_2$ (vitamin A_2 phenylazobenzoate using Karrer's open-chain formula)—C, 80.12; H, 7.74; N, 5.66; *Found*: C, 80.1; H, 7.8; N, 5.9. The compound had an ultraviolet absorption maximum at 341 mμ with E (1%, 1 cm) = 1,190. This shift to a lower wave length is due to the acid moiety, which has its own strong absorption band at 330 mμ.

Careful saponification under nitrogen of 258 mg of crystals gave 152 mg (theory, 150 mg) of vitamin A_2 alcohol. Adsorption on a small column of zinc carbonate showed a single homogeneous band with the exception of some strongly adsorbed red material (2 mg) at the top of the column and a tiny, weakly adsorbed orange band (about 1 mg) at the bottom of the column. These impurities were removed, and the vitamin A_2 eluted from the rest of the column was considered to be pure.

This preparation was a viscous, orange-yellow oil which showed two absorption maxima in the ultraviolet, one

[1] Communication No. 133 from the Laboratories of Distillation Products, Inc., Rochester, New York.

at 351 mμ with E = 1,460 and a subsidiary peak at 287 mμ with E = 820 (Fig. 1). A chloroform solution of $SbCl_3$ gave a blue color with a single absorption maximum at 693 mμ with E = 4,100 (Fig. 2).

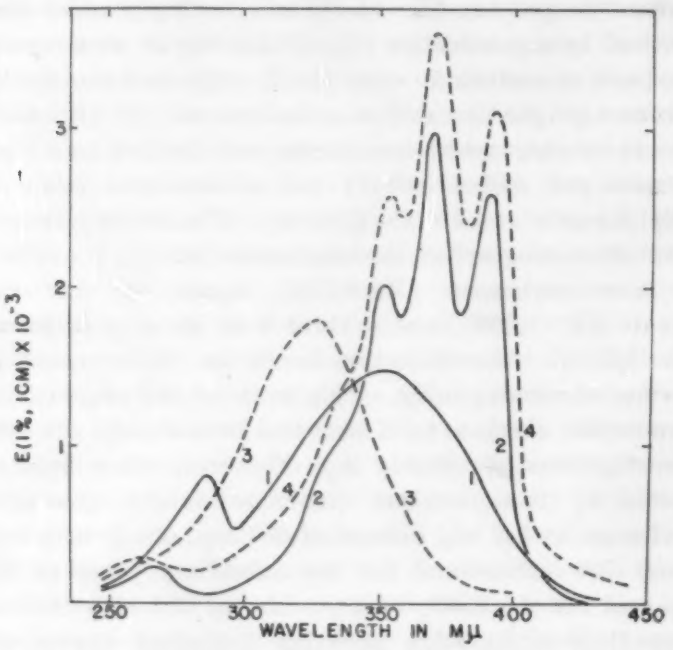


FIG. 1. Ultraviolet absorption spectra in ethanol of pure vitamin A_2 alcohol (curve 1) and crystalline anhydro vitamin A_2 (curve 2) compared with those of crystalline vitamin A_1 alcohol (curve 3) and crystalline anhydro vitamin A_1 (curve 4).

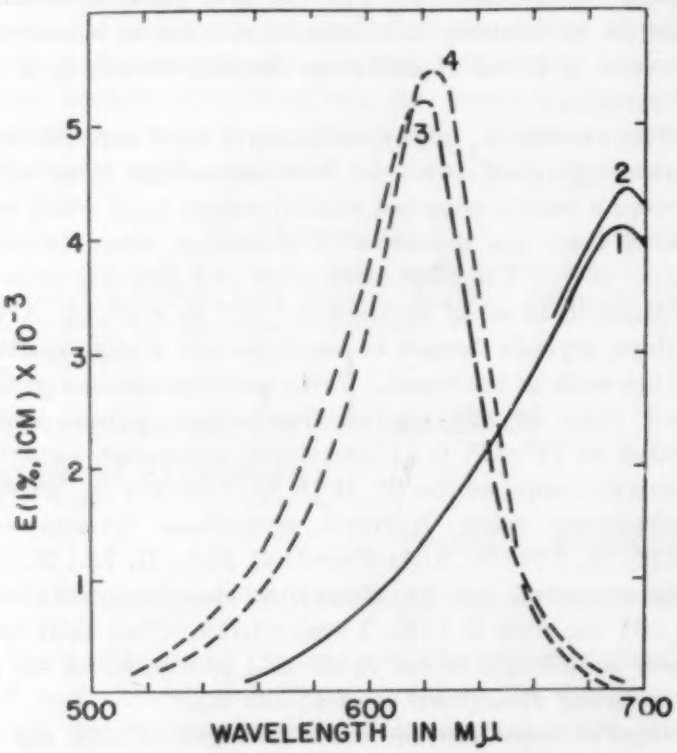


FIG. 2. Absorption spectra of the antimony trichloride products of pure vitamin A_2 alcohol (curve 1) and crystalline anhydro vitamin A_2 (curve 2) compared with those of crystalline vitamin A_1 alcohol (curve 3) and crystalline anhydro vitamin A_1 (curve 4).

Much of the existing confusion over the biological activity of vitamin A_2 has arisen from the fact that the $SbCl_3$ blue product of vitamin A_2 has appreciable absorption of its own at 620 mμ, although the $SbCl_3$ blue product of vitamin A_1 has very little absorption at 693 mμ (Fig. 2). Some workers have erroneously attributed th 620-mμ absorption of vitamin A_2 to vitamin A_1. In a earlier paper (*7*) the figure 3.06 was proposed as th ratio of E(693 mμ)/E(620 mμ) for vitamin A_2 prepar tions considered to be free of vitamin A_1. In the pu vitamin A_2 alcohol prepared above, this ratio was actual found to be 3.05.

Some of the slightly less potent vitamin A_2 alcoh fractions obtained in this work were combined and treate with N/30 alcoholic HCl to form anhydro vitamin A a hydrocarbon analogous to anhydro vitamin A_1 (*12*). Although vitamin A_1 and A_2 alcohols are extremely di ficult to separate chromatographically, their anhydr derivatives are readily separable (*2*). In the purificati of the above anhydro vitamin A_2 concentrate by adsor tion on zinc carbonate, no trace of anhydro vitamin A was found, showing the reliability of the separation livers under ultraviolet light.

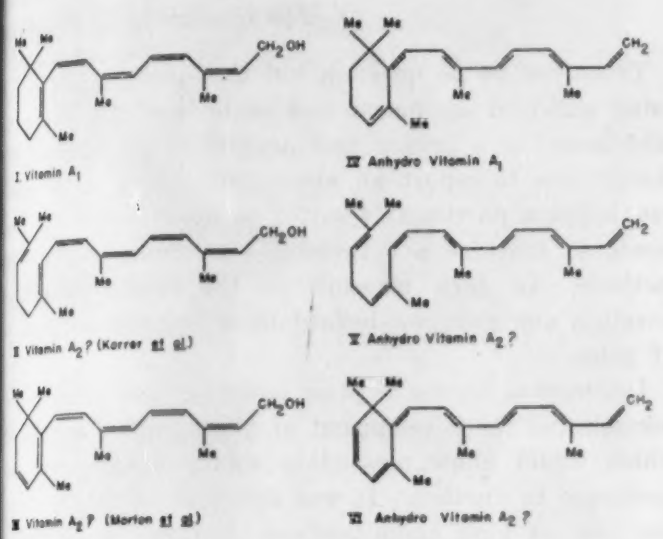
The chromatographically concentrated anhydro vitam A_2 was readily crystallized at −30° C from a 20% sol tion in light petroleum (Skellysolve "F"). Two r crystallizations showed no change in melting point (89.5 C, uncorr.). *Analysis*: required for $C_{20}H_{28}$ (open-cha formula)—C, 89.49; H, 10.51. *Found*: C, 89.2; H, 10. Microhydrogenation in an apparatus described by th author (*12*) showed the presence of 7 double bonds. Th ultraviolet absorption spectrum of this compound is a most identical with that of crystalline anhydro vitamin A (Fig. 1) with maxima at 352, 370, and 391 mμ havin extinctions of 2,040, 2,980, and 2,620, respectively. Th antimony trichloride blue color, however, is the same that of the original vitamin A_2 alcohol with an absor tion maximum at 693 mμ and a slightly higher extinctio of 4,400 (Fig. 2).

The above data shed but little light on the structur of vitamin A_2. The first proposed structure of Gillam *et al.* (*3*), containing an extra vinylene group in the vit min A chain, has been discarded on the basis of th distillation data of Gray and Cawley (*5*). Two othe possibilities of merit are the open-chain formula Karrer, *et al.* (*9*), relating vitamin A_2 to vitamin A_1 lycopene is related to β-carotene, and the formula Morton, *et al.* (*11*), containing a dehydroionone rin Karrer's proposal is based chiefly on oxidative studies vitamin A_2 concentrates, while Morton's is based on th claim that the "C_{20} aldehyde" of Haworth, *et al.* (*6*) obtained by Oppenauer oxidation of vitamin A_1 wit diethylketone, is, in fact, the aldehyde of vitamin A_2 an is convertible to vitamin A_2 *in vivo*. This compound supposedly the aldehyde of compound III.

Meunier, *et al.* (*10*) have postulated the structure anhydro vitamin A_1 to be that given as formula IV This possibility was given in a paper from this laborator at about the same time (*13*), and the author is in agre ment with this proposal. If such is the case, then b analogy the corresponding structure for anhydro vitami A_2 would be formula V if structure II is correct an formula VI if structure III is correct.

The fact that the two anhydro vitamins have almo identical ultraviolet absorption spectra but give totall

different blue colors has been disregarded by most investigators, although an account of this seeming anomaly was first published in 1940 (*2*). Compounds IV and VI would hardly have the same ultraviolet spectra, but com-


I Vitamin A1
II Anhydro Vitamin A1
III Vitamin A2? (Karrer et al.)
IV Anhydro Vitamin A2?
V Vitamin A2? (Morton et al.)
VI Anhydro Vitamin A2?

pounds IV and V might be very similar. The surprisingly large difference in the blue colors could then be accounted for by assuming that antimony trichloride brings the isolated double bond of compound V into play. On the other hand, the shift in ultraviolet absorption maximum of compound II to 351 mμ from that of vitamin A_1 (I) at 326 mμ is rather large to be accounted for by mere opening of the ring. In the author's opinion, the evidence slightly favors structure II for vitamin A_2, if a choice must be made between these two proposals.

Biological tests indicate that the vitamin A_2 alcohol described above has a potency of approximately 1,300,000 U.S.P. units/gm, or about 40% of the activity of crystalline vitamin A_1. The biological experiments will be reported in greater detail elsewhere.

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Some Quantitative Aspects of the Feulgen Reaction for Desoxyribose Nucleic Acid

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The Feulgen reaction, originally described by Feulgen and Rossenbeck (*2*), has been used for the demonstration of desoxyribose nucleic acid (or thymonucleic acid) in tissue sections. The reaction involves the hydrolysis of sectioned material in 1N HCl at 50–60° C for about 10 min, followed by the application of the leuco-fuchsin (Schiff's) reagent for 1–2 hrs. Stowell (*5*) and Dodson (*1*) presented strong evidence that the reaction is specific for thymonucleic acid. The Feulgen technique has been applied in various ways for the quantitative estimation of the desoxyribose nucleic acid (DNA) in the cell. In order to establish its possible value as a quantitative method, the Feulgen reaction was tested on purified preparations of DNA.

It was impossible to separate the components of the reaction in liquid medium; therefore, it was necessary to find a solid medium into which the DNA could be incorporated. Weighed amounts of DNA were dissolved in hot 5% agar as previously described by Hillary (*3*). Eight different samples of agar without DNA, however, gave a positive Feulgen reaction following the usual hydrolysis. The fixatives used by Hillary were tried, but none was able to inhibit the Feulgen reaction with hydrolyzed agar. It became evident that Hillary's methods involving agar were unsatisfactory and that his conclusions on the effect of fixatives on the Feulgen reaction were open to question.

The problem was to find a medium that would gelate or solate at appropriate temperatures and which, after fixation and hydrolysis, would be negative to the Feulgen reaction. Eastman Kodak ash-free and Difco-Bacto gelatin were satisfactory. After fixation of a 20% gel in 6% formalin for 1½ hrs, there was no appreciable hydrolysis of the gelatin by the HCl. The gelatin was Feulgen negative after long periods of hydrolysis provided the formalin was thoroughly washed out. Formalin itself gives an intense Feulgen reaction even at very low concentration. Sodium DNA, at the concentrations used, readily dissolved in the 20% gelatin sol and was not appreciably lost from the gel during fixation, washing, hydrolysis, and the subsequent reaction with the leuco-fuchsin reagent (prepared by the Rafalko technique, *4*).

The procedure was to add 5 cc of the 20% gelatin sol, at 32–35° C, to small test tubes containing weighed amounts of sodium DNA. Careful agitation produced a uniform mixture of the gelatin sol with the DNA. Drops of the warm gelatin-DNA preparations, approximately 0.1 ml in volume, were then placed on 1″ × 3″ glass slides (previously coated with albumin fixative and heat dried) and allowed to gel. The gelatin drops were hardened in 6% formalin for 1½ hrs and washed in running tap water for 2–3 hrs to remove the formalin. The

preparations were considered to be formalin free if, after a 30-min period in the leuco-fuchsin reagent, the test slide remained completely colorless.

With this procedure, gelatin preparations containing DNA at 0.2, 0.5, 0.8, 1.0, 1.5, 2.0, and 3.0 mg/cc were tested. One drop of each preparation together with two or more DNA-free control drops were placed on a slide. These slides, each representing a test series, were hydrolyzed in 1N HCl at 55° C for from 3 to 39 min and then immersed for 1½ hrs in the leuco-fuchsin reagent. Color estimations were made after a quick rinse in distilled water and then two 10-min changes of water saturated with SO_2. The data from several test series are summarized in Table 1.

TABLE 1

Conc. DNA (mg/cc)	Hydrolysis in 1N HCl at 55° C (min) 0	3	5	7	9	11	13	15	17	19	22	27	34	39
0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0	0	0	1	0	0	0
0.8	0	0	0	0	1	2	2	1	2	3	2	1	1	2
1.0	0	0	0	1	1	2	2	1	1	2	3	3	3	3
1.5	0	1	1	2	2	2	3	4	4	4	4	4	4	
2.0	0	1	1	2	2	3	3	4	4	4	4	4	4	
3.0	0	1	1	2	2	3	3	4	4	4	4	4	4	

The values indicate the relative intensity of color developed in the gelatin drop: 0 = absence of color or, at most, a faint tinge, 1 = light lavender, 2 = lavender, 3 = light purple, 4 = deep purple.

No appreciable color was developed with small amounts of DNA, while within the middle range there was a gradual increase of color until a maximum intensity was reached. It should be pointed out that there was no further development of color with increasingly greater amounts of DNA. The intensification of the color of the drops that develops with increasingly longer periods of hydrolysis indicates that DNA was not lost in the process. With higher concentrations of DNA there was no increase or decrease in color intensity following periods of hydrolysis beyond those required for maximum color development. These observations indicate the necessity for a critical control of hydrolytic time.

The Feulgen reaction is quantitative only over a short range of DNA concentrations. At least 0.8 mg/cc of DNA must be present in order to produce an appreciable color. Beyond a concentration of 1.5 mg/cc no further intensification of color may be visually detected after an hydrolysis of 15 min or longer. Experiments are in progress to study the reaction by photometric methods.

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Photomicrography at Your Convenience[1]

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There can be no question but that photomicrography using standard equipment and methods of the biological laboratory, is a proven and acceptable procedure. We should like to report an alternative which, after some use, appears particularly suited to our needs and which combines features not found in the more widely used methods. In such measure as the reader's problem parallels our own, we believe these suggestions will be of value.

Intefests so diverse as plant pathology and cytogenetics necessitated the development of photographic equipment which would allow reasonably rapid change from one technique to another. It was desirable to take pictures not only at high magnifications through the compound microscope, but through the dissecting microscope, and at low magnifications by use of extension tubes.

One camera, the Kine Exakta 35-mm single lens reflex seems ideally constructed for the purpose. Especially valuable features of this machine are: attached magnifier for use with ground-glass focusing, shutter speeds from .001 to 12 sec, bayonet lens mount, focal plane shutter, and provision for removal of short lengths of exposed film. Except for the added light intensity obtainable for focusing, there is little advantage of the f 2.8 over the f 3.5 lens. This camera, if the tripod socket is fitted with the proper reducing insert, can be attached to the horizontal arm of any enlarger stand either by the bolt used to hold the enlarger unit itself or by an ordinary ¼″ bolt of the proper length and of standard thread. For maximum speed and accuracy it is well to calibrate the vertical post of the enlarger stand in convenient units and to fix the height of the camera at will by use of a radiator hose coupling about the enlarger post. This hose coupling, if selected of the proper diameter, can readily be moved from one position to another, allowing the arm to swing freely in the horizontal plane (Fig. 1).

Probably the most effective way of joining the camera to the barrel of the microscope is by use of a 6″-8″ length of automobile radiator hose, which may be obtained in a size which snugly fits the extension tube. By slipping the rubber sleeve up over the metal tube, the eyepiece of the microscope is cleared, and the camera can be swung to one side. By slipping it down over the barrel of the microscope, an essentially lightproof seal is secured. The manipulations are completed by fixing the enlarger stand firmly to the working desk and marking the position of the base of the microscope and lamp.

Exposure time, inevitably a matter of trial and error when once established, will be constant. The complexity of the lamp and optical equipment available will deter

[1] Contribution No. 102 from the Botanical Laboratory of the University of Tennessee.

e the exact details, but in general it is necessary to ndardize the diaphragm openings of both microscope ndenser and light source, rheostat setting of the lamp,

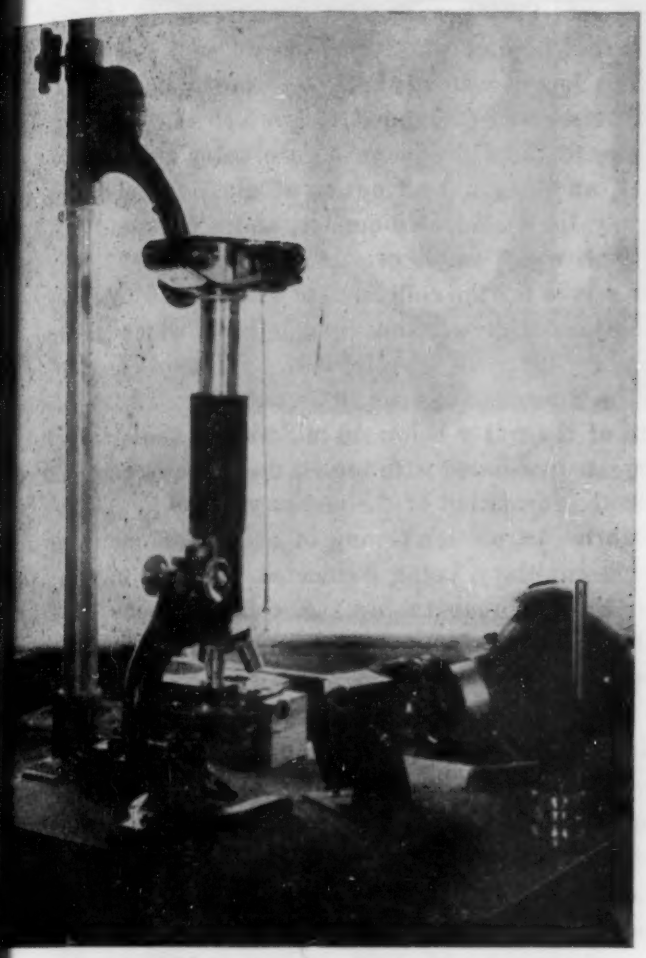

FIG. 1. The camera in position for taking a photomicrograph.

ance from lamp to microscope mirror, and height of era above the eyepiece. Once this is done, it is no blem to establish proper exposure times for the sev- desired magnifications. Choice of film, filters, etc. matters best left to the whims of the individual earcher.

Perhaps the only disadvantage of this apparatus is the h initial cost of the camera itself. We feel that this more than offset by the following:

(1) Photomicrographs can be so quickly and effecly taken that the newly popular smear techniques in ology are fully applicable. One can work at the roscope unimpeded, photographing with expenditure but a few minutes whatever seems of interest.

(2) Cost of film is sufficiently low that large numbers photomicrographs can be taken and records kept in form, rather than as drawings or as stage vernier dings. There is decidedly less need for maintaining ge stocks of old preparations.

(3) Kodachrome slides can be directly produced, and ×2″ black-and-white transparencies can be made simby contact printing on a high contrast ortho film. that matter, negatives of larger sizes can be photoed by transmitted light and reproduced as 2″×2″ nsparencies.

(4) The same camera can be used for photomicrographs, for close-up photographs through dissecting microscope or extension tubes, for copying illustrations or microfilming books, and for ordinary work in the field. Frequently not even a change of film is necessary.

Use of the Dropping-Mercury Electrode for the Continuous Measurement of Dissolved Oxygen in Flowing Water[1]

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In connection with measurements of the basal metabolic rates of fishes, it was found desirable to obtain continuous records of the oxygen content of a stream of water issuing from the metabolism chamber. Giguère and Lauzier (*1*) have described a polarographic method for measuring the dissolved oxygen in flowing water, but the conditions necessary for its performance make it unsuitable for the present application. The polarized dropping-mercury electrode seemed to be more promising, particularly as employed by Manning (*3*), who obtained a continuous 24-hr record of the oxygen content of unmodified lake water. However, because of progressive changes in the surface of the quiet pool of mercury (the anode), this method could not be made to yield satisfactory results when applied to flowing tap water.[2] Although the oxygen content of the water did not change, the limiting current at constant voltage (1 v) decreased steadily throughout all trials. A calomel electrode with saturated KCl solution and an agar bridge (*2*) performed somewhat better, but the results were still not satisfactory, the limiting current continuing to diminish. A sealed calomel electrode with a fiber bridge gave the same result. Apparently the concentration of the salt across the bridge, or the pressure within it, undergoes a progressive change while the electrical current is flowing which alters the potential across the bridge.

The difficulty can be overcome by opening the bridge to an open reservoir containing a saturated solution of KCl and calomel, the reservoir being set at such a level that the pressure of the solution on the bridge is slightly greater than that driving water into the electrolysis cell. A pressure of a few centimeters of water is sufficient. Under these conditions there is an extremely slow flow of saturated solution through the bridge into the electrolysis cell (less than 0.4 ml in 24 hrs), so that there is no change in potential across the bridge. With this modification of the calomel electrode, the limiting current remains constant for extended periods, sometimes for as long as a week. Experimental changes in the oxygen content of the water flowing through the cell are accompanied

[1] Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

[2] Charcoal-filtered, aerated city water.

by corresponding changes in the limiting current over the range of concentrations tested so far (2.3–7.4 mg/liter). By use of recording equipment[3] it is possible to obtain continuous records of the oxygen content of running tap water for long periods. Unless records are desired, the combination of a Weston cell and galvanometer, described by Petering and Daniels (*5*), is very satisfactory for continuous operation.

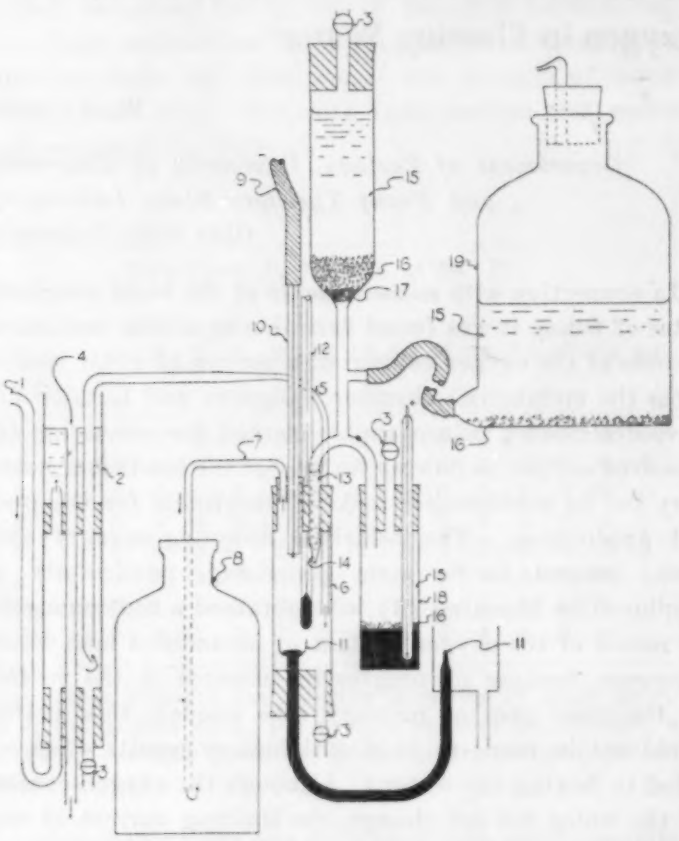

FIG. 1. Assembly of apparatus: 1, water inlet from metabolism chamber; 2, constant-level reservoir (glass tubing 20 × 115 mm); 3, stopcocks (closed during operation); 4, overflow; 5, inlet to electrolysis cell; 6, electrolysis cell (glass tubing 20 × 65 mm); 7, outlet from electrolysis cell; 8, constant-level overflow bottle; 9, Neoprene tubing from mercury reservoir; 10, capillary; 11, mercury overflow; 12, thermometer; 13, 3% agar gel in saturated solution of KCl and calomel; 14, opening from agar bridge; 15, saturated KCl and calomel; 16, KCl crystals; 17, glass-wool retainer; 18, calomel electrode; 19, reservoir for bridge.

It is necessary to control the flow rate of the water, the pressure of the mercury in the capillary, the pressure of the saturated solution on the bridge, and the temperature, as variations in these cause the limiting current to vary in the same direction. The changes in the limiting current are relatively small, however, so that when it is desired merely to indicate the approximate concentration of oxygen in the water (to within a few tenths of a milligram/liter), the assembly requires very little attention. For precise measurements it is necessary to maintain nearly constant conditions throughout the run. The assembly shown in Fig. 1 has been found to be quite satisfactory for this purpose. Although the diagram is self-explanatory for the most part, several features require further description. In general, the techniques described by Kolthoff and Lingane (*2*) are employed; only those more or less peculiar to the present assembly are described here.

The flow of water is held constant by use of a constant-level reservoir (arranged to protect the water from exposure to the atmosphere before entering the electrolysis cell), an inlet and an outlet of glass tubing of approximately 1.5-mm inner diameter, and a constant-level bottle through which water overflows from the cell. The bottle also serves for the collection of samples for Winkler determinations of dissolved oxygen, against which the dropping mercury electrode is calibrated. With an electrolysis cell of the dimensions given, it is necessary to keep the flow rate of the water below 40 ml/min, as the stronger water currents associated with higher flow rates seem to interfere with the formation of the mercury drops.

Marine barometer tubing of about 0.05-mm bore serves as the capillary, being connected with the mercury reservoir by Neoprene tubing. A constant head of pressure (about 60 cm of mercury) is maintained by the method described by Mueller (*4*). The drop rate is set at about 15 drops/min, at which rate approximately 12.5 ml of mercury pass through the capillary in 24 hrs. It is necessary to clean the tip of the capillary from time to time in order to prevent irregularities in the drop rate, but as a rule it can be operated continuously for from four days to a week before these irregularities appear. Under ordinary conditions of operation, this is the only part of the assembly that requires attention.

The electrolysis cell is enclosed in a water jacket, not shown in the diagram, through which water is kept circulating to prevent changes in the temperature.

Although it should be held reasonably constant, the pressure of the saturated solution on the bridge may be permitted to vary by a few millimeters of water in the course of a run without affecting the results appreciably. As the flow of solution through the bridge is very slow, the pressure change in the course of a week can be made negligible by use of a large reservoir.

The glass tube containing the agar plug is drawn to a narrow tip, with an opening about 1 mm in diameter on one side, about 1 mm back from the blind tip. In this manner the occasional air bubbles and debris that find their way into the system are prevented from accumulating under the agar connection, being swept by it in the water current.

[3] Recording was accomplished by means of a Leeds and Northrup "Electrochemograph," with the polarizing unit set at −1 v and the chart speed reduced.

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